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Editorial

We are pleased to announce the new issue of Aurum Journal of Health Sciences (AJHS-A.J.Health Sci.) has been published. We would also like to announce that the efforts to increase number of the articles and to improve the quality of the journal have gained speed. With this issue, article submission and reviewer evaluation processes will be carried out through DergiPark online platform. The aim of this adjustment is to facilitate those processes for our writers and reviewers, to standardize the steps, to increase the visibility of the journal and to reach to a large number of readers.

One of our main goals is to ensure full adherence to the ethical rules regarding publishing. It is aimed to improve the editorial policy and develop it in accordance with the standards in the international guidelines. Our studies in this direction will enhance our publication quality and increase our chances of being in national/international indexes.

A.J.Health Sci is published tri-annually (January-May-September) as a peer-reviewed health sciences journal and targets to provide a platform for health science research with interdisciplinary discussions. In this issue, we published articles in dental sciences and forensic medicine. I would like to thank all the writers and reviewers who contributed to this issue and also to thank our editor, Associate Professor Başak Bıyıkoğlu, who made a great contribution in the field of dentistry. I wish a great year with full of success for our journal, AJHS.

Gaye Hafez, Ph.D

Editor in Chief

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Research Article

A Comparative Analysis of Biofilm Characteristics of Dual-Species Periodontopathogenic Biofilm based on Fusobacterium nucleatum and the Dual-Species Biofilm Response in the Presence of Antimicrobial Peptide

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Abstract: *Fusobacterium nucleatum (F.nucleatum)*, which acts as a linking periodontopathogenic bacteria, has the ability of coaggregation with early and late colonization species. It is the most intense gram-negative bacteria found in periodontal health and increases in periodontal diseased areas numerically. In this study, comparative analyses of dual and mono-biofilm structural characteristics formed by *Porphyromonas gingivalis* and *Prevotella intermedia* with *F. nucleatum* have been evaluated. In all results, more biofilm formation was observed in dual-species compared to mono-species biofilms. Biofilm formation of *F. nucleatum-P.intermedia* has increased in the presence of Human Neutrophilic Peptide (HNP-1). In this study it is concluded that dual-periodontopathogenic coaggregations based on *F. nucleatum* could produce more intense biofilm structure than single-species ones, and the increase in dual-species biofilm mass in the presence of low doses of HNP-1 may be a defense mechanism of dual periodontopathogenic biofilm.

Keywords: *Periodontitis; subgingival; biofilm; antimicrobial; peptide; gram-negative bacteria; defensin; inflammation*

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1. Introduction

Bacteria within the oral cavity are organized as multi-species biofilms. These structures containing around 500-700 species play an important role in the development of caries and periodontal diseases. Periodontitis is a biofilm induced bacterial inflammatory disease and characterized by progressive destruction of tooth supporting tissues. It is known that bacteria in biofilm have different characteristics compared to their planktonic life (Walker et al., 2007). There are studies showing that bacteria in biofilm can possess a more resistant structure against variables such as antimicrobial agents, physical forces, and pH changes (Shaddox et al., 2010). It has also been reported that multi-species biofilm structure may exhibit more

pathogenicity than single-species bacterial infections (Ebersole et al., 2017; Lamont et al., 2015; Lamont et al., 2018). Gram-negative bacteria are etiologically the most common species in the development of periodontitis. Among these, *F. nucleatum* plays a key role in the formation of dental biofilms and is involved in the association of early and late colonization types, including *P. gingivalis* and *P. intermedia*, which play important role in the progression of periodontitis (Bolstad et al., 1996; Kolenbrander et al., 1993).

The Cationic Antimicrobial Peptides (CAMPs), important elements of the host defense system against the presence of infectious agents, belong to two families in human, namely defensins and cathelicidins. CAMPs act as a broad spectrum antimicrobial and also involve in immune system stimulation. HNP-1 has been shown to be the most abundant one among the 4 types of defensins isolated from neutrophilic granules (Keskin et al., 2014; Lundy et al., 2008). Bacteria and defensins are constantly present in the mouth. According to study of Keskin M. et al. (2014) it was concluded that *F. nucleatum* could develop some adaptation responses to the presence of defensins. The aim of this study is to investigate the dual-species biofilm characteristics of *F. nucleatum* with *P. gingivalis* and *P. intermedia* and also the dual-species biofilm characteristics in the presence of low concentration of HNP-1.

2. Materials and Methods

2.1. *Bacterial Species and Cultures*

In all laboratory experiments, type strains of *F. nucleatum*, *P. gingivalis* and *P. intermedia* (*F. nucleatum* ATCC 25586, *P. gingivalis* ATCC 33277, *P. intermedia* ATCC 25611) were used. All bacteria were incubated in hemin (5 mg/l) and vitamin K1 (10 mg/l) supplemented Brucella agar at 37°C in 10% H_2, 5% CO_2, and 85% N_2 anaerobic atmosphere (Whitley A35 Anaerobic Workstation, Don Whitely Scientific Ltd., West Yorkshire, UK) for 72 hours. Mature colonies were collected with sterile cotton swabs and transferred to KH_2PO_4 (final concentration: 25 mM), $MgSO_4$ (final concentration: 4 mM) and saccharose (final concentration: 1%) supplemented Bactoetryptone specific broths and incubated for 48 hours under anaerobic conditions. Optical densities were standardized to 0.6_{OD} at 490 nm just before the experiments.

2.2. *Experimental Groups*

The following seven different groups were designed;

Group A: *F. nucleatum* alone: 180 µl bacterial growth media + 20 µl *F.nucleatum*

Group B: *P. gingivalis* alone: 180 µl bacterial growth media + 20 µl *P.gingivalis*

Group C: *P. intermedia* alone: 180 µl media + 20 µl *P.intermedia*

Group D: *F.nucleatum* +*P.gingivalis* (Dual): 180 µl media + 10 µl *F.nucleatum* + 10 µl *P.gingivalis*

Group E: *F.nucleatum* + *P. intermedia* (Dual): 180 µl media + 10 µl *F.nucleatum* + 10 µl *P.intermedia*

Group F: *F.nucleatum* + *P. gingivalis*. (Dual+HNP): 180 µl media + 10 µl *F.nucleatum* + 10 µl *P.gingivalis*. + 5 µg/ml HNP-1)

Group G: *F.nucleatum* + *P. intermedia* (Dual+HNP): 180 µl media + 10 µl *F.nucleatum* + 10 µl *P.gingivalis* + 5 µg/ml HNP-1)

All experiments were performed in triplicate wells for each condition and repeated at least twice. Samples were taken from all the wells and placed on Brucella agar for contamination control.

2.3. *Preparation of Defensin Suspension*

HNP-1 was purchased commercially (HNP-1/Code: 4271-s Lot: 610505, Peptide institute, Japan) and stored at -20°C until use. HNP-1 was dissolved in bacterial growth media on the day of the experiment and prepared as 5 µg/ml concentration.

2.4. *Preparation of Saliva Coated 96-well Plates*

Clarified Saliva were prepared and stocked before all experiments. Salivas were collected from healthy male and female volunteers to 50 ml propylene tubes and centrifuged at 12,000 g at 4°C for 40 minutes. The supernatants were collected and divided into sterile glass tubes. The tubes were incubated for 30 minutes at 60°C for pasteurization and then centrifuged again at 12,000 g and stored at +4°C until use. 20 µl of each supernatant was collected and placed on Brucella agar, then incubated under aerobic and anaerobic conditions for 4 days and sterilization controls were performed. 50 µl of saliva was added to all the wells and plates were incubated for 1 hour at 37°C. All wells were then dried without washing and prepared for the experiment.

2.5. *Analysis of Biofilm Mass Formation*

Biofilm mass formation tests were performed by Crystal Violet Staining Method in all groups (Keskin et al., 2014). All wells were stained with 0.1% Crystal Violet. After incubation at room conditions for 15 minutes, the wells were washed twice with PBS (Phosphate Buffer Saline) to remove excess dye. 200 ml of acetic acid was added to each well and incubated for 10 minutes to allow homogenous distribution of Crystal Violet dye. Afterwards, spectrometric analysis (570 nm) was performed on all plates.

2.6. *Analysis of Biofilm Protein Content*

The incubated plates were washed with PBS and dried. At the end of these washing and drying processes, wells free from planktonic bacteria and liquid medium were filled with 100 ll of 0.2 N NaOH. 2 sec/ 80 W sonication (dr hielscher UP50H homogenizer) was applied to each well to ensure homogeneous distribution of the present biofilms at the bottom (Lu et al., 2007). Afterwards, the plates were transferred to a microwave oven operated at 600 W for 20 seconds (Akins et al., 1995). Then, BIO-RAD protein assay dye was added to each well at a rate of 1/4 of the total volume (25 µl). Following the instructions for the

BIO-RAD kit manual, plates were incubated for 5 minutes at room temperature. Then absorbances were measured at 595 nm by spectrophotometer (Thermo Labsystems x-355) and the OD value was recorded.

2.7. *Analysis of Biofilm Polysaccharide Quantity*

FITC-labeled Concanavalin A (FITC-Con A) fluorescent dye was used for fluorometric analysis of biofilm polysaccharide content. Washing and drying of 96-well plates was carried out for twice with PBS. Each well was filled with 100 µl of FITC-Con A fluorescent dye (concentration of 50 µg/ml) and incubated at room temperature in a dark room for 5 minutes. After incubation, plates were washed twice with PBS and dried. The 96-well plates were analyzed and recorded by using the BIOTEK Synergy HT Fluorometer Instrument (Excitation=485, Emission=528).

2.8. *Statistical Analysis*

In dual-species biofilm tests, single bacterial values were accepted as constant, and each of different dual-species biofilm structures was accepted as a variable. Paired-sample parametric t-test was used to evaluate whether there was a difference in the biofilm structures of any group. P values <0.05 were considered statistically significant. In defensin tests, dual-species biofilms with HNP-1 free media were accepted as constant value and the wells with HNP-1 were accepted as variable and paired-sample parametric t test was applied for statistical analysis.

3. Results

P. gingivalis- F. nucleatum dual-species biofilms produced more biofilm mass, protein and polysaccharide synthesis compared to all mono-species biofilms (Figure 1, 2 and 3) ($p < 0.05$). Similar results have been observed in the dual-species biofilms of *P. intermedia* and *F. nucleatum* (Figure 1, 2 and 3) ($p < 0.05$). Statistically significant polysaccharide synthesis was found in *P. gingivalis- F. nucleatum* and *P. intermedia - F. nucleatum* dual-species biofilm structures compared to mono-species biofilms (Figure 3) ($p < 0.05$). It was observed that *F. nucleatum- P. intermedia* dual-species biofilms were able to produce statistically significant amounts of biofilms in the presence of HNP-1 compared to HNP-1 free media (Figure 4) ($p < 0.05$). All other dual-species biofilm tests with HNP-1 showed no statistically significant difference (Figure 5 and 6).

Figure 1. Dual-Biofilm Formation (Crystal Violet)

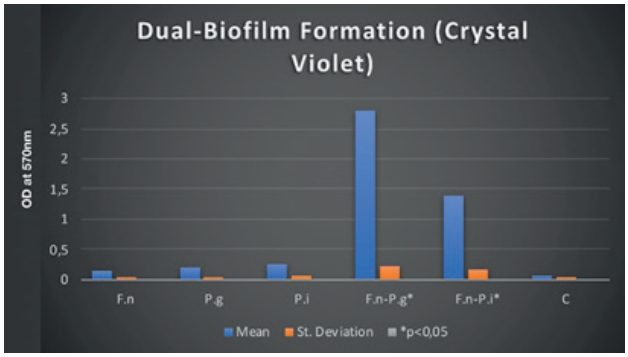


Figure 2. Dual-Biofilm Formation (Protein)

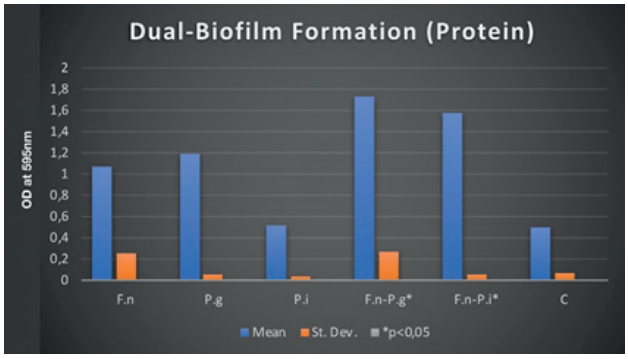


Figure 3. Dual-Biofilm Polysaccharide Production

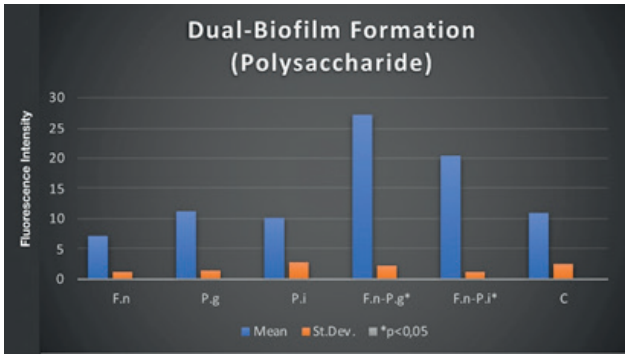


Figure 4. Dual-Biofilm + HNP-1 (Crystal Violet)

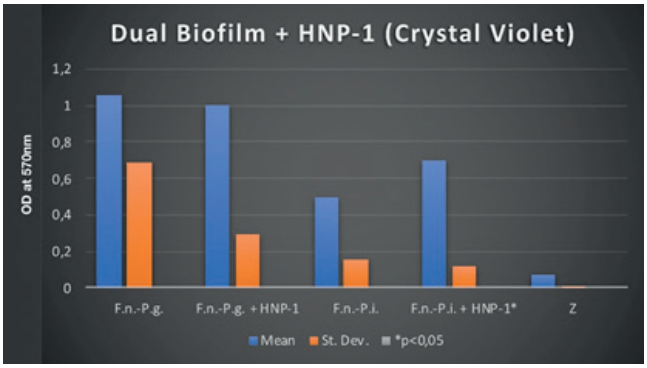


Figure 5. Dual-Biofilm + HNP-1 (Protein)

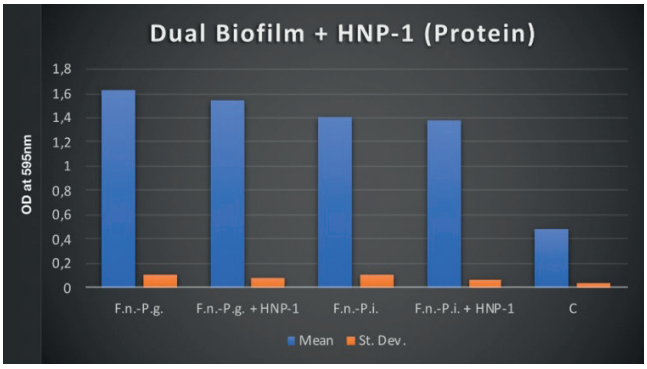
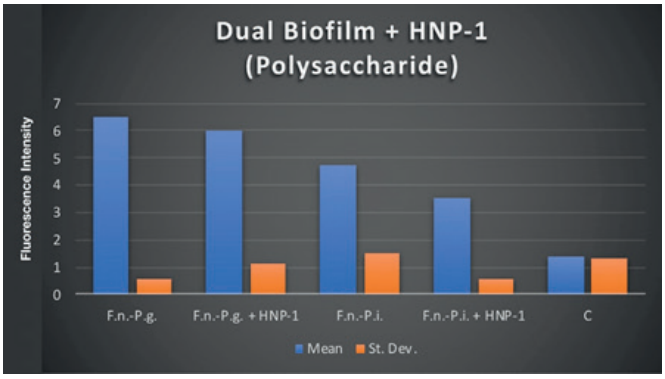


Figure 6. Dual-Biofilm + HNP-1 (Polysaccharide Production)



4. Discussion

Gram-negative, anaerobic bacilli types are most closely associated with periodontitis. Among these, *F. nucleatum*, *P. intermedia*, *P. gingivalis* are the most common bacterial species and play a key role in the progression of the disease (Socransky et al., 1998; Teles et al., 2010).

In this study, characteristics of dual-species biofilms formed by *P. gingivalis* and *P. intermedia* with *F. nucleatum* were investigated. In order to grow them, incubation time of the bacteria in anaerobic conditions was determined as 48 hours for biofilm formation experiments. In previous studies it was shown that anaerobic incubation period of 48-72 hours could provide the appropriate time for the biofilm formation of *F. nucleatum* (Jang et al., 2013; Saito et al., 2008). The Crystal Violet technique was used in the analysis of biofilm mass after incubation. Because it is known as a reliable method and has been used in many studies (Merritt et al., 1998; Pitts et al., 2003; Stepanovic et al., 2000). Bradford Protein Analysis was also included for comparative analysis of total biofilm mass and biofilm protein values. While performing Bradford Protein Analysis, sonication was applied to all the wells to ensure homogeneous distribution of the biofilm mass (Bradford et al., 1976; Lu et al., 2007). FITC - Con A fluorescent dye was used to determine the amount of biofilm polysaccharide. FITC labeled Concanavalin A is a fluorescent fluorophore substance that binds with polysaccharide and is frequently used in *in vitro* polysaccharide studies (Roth et al., 1978; Sato et al., 2006; Yang et al., 2006).

The *P. gingivalis* and *P. intermedia* dual-species biofilms formed by *F. nucleatum* showed statistically significant increase in biofilm mass, polysaccharide synthesis and protein ratios when compared with mono-species biofilms. All these results show that *P. gingivalis* and *P. intermedia* dual-species biofilms formed with *F. nucleatum* are able to produce intense biofilms compared to single-species biofilms synergistically. Based on previous studies, it was observed that bacterial coaggregation plays an important role in biofilm formation. Similar to the results of this study, there are many other studies showing that *F. nucleatum* could play an important role in binding of late colonization species within the biofilm (Kolenbrander et al., 1989; Kolenbrander et al., 1993; Rickard et al., 2003).

In this study, dual periodontopathogenic biofilm characteristics were analyzed in the presence of HNP-1 comperatively. Other studies show that the HNP-1 concentration in the oral cavity can be found in a wide range of values from 0.40 µg/ml to 100 µg/ml (Dale et al., 2006; Fanali et al., 2008; Goebel et al., 2000; Puklo et al., 2008). However, there is a finding which indicates no lethal effect at low concentration levels of HNP-1 on *F. nucleatum* (Miyasaki et al., 1998). Therefore, the concentration of HNP-1 (5 µg/ml), which can be found in oral cavity continously, was studied to evaluate the defensive response of dual-species biofilms to HNP-1 challenge. It was observed that *F. nucleatum - P. gingivalis* dual-species biofilm did not show any significant change of biofilm production in the presence of HNP-1 compared to defensin-free media. Additionally, it was seen that the presence of HNP-1 did not lead to statistically significant difference in polysaccharide production at any biofilm group. It was formerly reported that dual-bacterial biofilms form an intense biofilm structure than single bacteria biofilms (Okuda et al., 2012; Saito et al., 2007).

Although *P. intermedia - F. nucleatum* dual-species biofilm showed a statistically significant increase in biofilm mass in the presence of HNP-1, the same increase was not observed in polysaccharide

values. Flemming et al. reported that the matrix structure of the biofilm occurs in products other than polysaccharides such as proteins, lipids and nucleic acids, as well (Flemming et al., 2010). Consequently, dual-periodontopathogenic coaggregations based on *F. nucleatum* could produce more intense biofilm structure than single-species ones, and the increase in dual-species biofilm mass in the presence of low doses of HNP-1 could act as a defense mechanism of *F. nucleatum- P. intermedia* dual-species biofilm. More enlightening studies are needed over this subject.

Conflict of interest

Author declares no conflict of interests.

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Review

Epigenetic Approach in Forensic Age Estimation

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Abstract: Age estimation study is a very important research area that contributes to the solution of the forensic case by helping to identify the identity in forensic sciences. Human age estimation in the traditional way is performed by analysis of bony marks on bones and teeth. An analysis of the age estimation of biological samples from the use of genetic analysis has not yet become part of routine practice. The use of genetic analyses for forensic purposes started with the Restriction Fragment Length Polymorphism (RFLP) analysis in the late 1980s and developed with Short Tandem Repeats (STR) analysis. Along with the technological developments in forensic genetics, progress has continued with single nucleotide polymorphism (SNP) analysis, which enables the identification of hair, eye and skin color and geographic infrastructure of an unknown sample in forensic case resolution. However, recent studies in forensic genetics have focused on epigenetic mechanisms and it has been discovered that DNA methylation can be used in case resolution for forensic age estimation. With the development of DNA methylation studies, a quantitative statistical relationship has been established between DNA methylation and different age groups. The results have been obtained with ± 3-4 age prediction accuracy using DNA methylation markers (CpG regions) tested to date with different methodological approaches. Thus, with the advancement of epigenetic studies in the fields of forensic sciences, the phenotypic features of the DNA of the evidence samples have been estimated with some error rates. The aim of this study is to reveal the latest developments in the field of epigenetics and evaluation of the use of epigenetic-based age estimates for forensic purposes.

Keywords: *Forensic genetic; epigenetic; DNA methylation; age estimation*

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1. Introduction

1.1. *Epigenetic and Age*

Age estimation is one of the important subjects of forensic sciences used to determine the identity of suspects. Age estimation of people with traditional methods, bone and teeth are made by the analysis of bony traces (Hillewig et al., 2013; Olze et al., 2006). However, this approach is limited to cases of a skeleton. On the other hand, biomarkers in which 4977-bp was detected in this area, which was removed from the mitochondrial DNA (mtDNA) at the molecular level (Meissner et al., 1997), d-aspartic acid ratios in proteins (Helfman et al., 1976) and length of leukocyte telomeres (Blasco, 2005) could be used to age estimation. However, the age estimates made by these biomarkers defined so far have significant limitations (because of the poor understanding of the mechanisms that cause the deletion of mtDNA) (Meissner et al., 2010).

Epigenetics is a rapidly developing field as a result of new technological developments and the increase of biological discoveries. The relationship between epigenetic and environmental factors is not well defined in mammals, however, it was found that hypo and hypermethylation levels in the methylation regions of DNA were related to aging (Day et al., 2013; Fraser et al., 2012; Tammen et al., 2013). Thus, DNA methylation levels can be used in case of solutions for forensic age estimation. Researchers working on this subject have discovered the age-related specific methylation sites in the human genome with aging. In this context, it has been observed that forensic sciences experts have increased their interest in age-related DNA methylation markers in the last decade. ±3-4 year prediction accuracy results were obtained from biological samples using DNA methylation markers (CpG sites) tested to date with different methodological approaches. Thus, with the advancement of genetic and epigenetic studies in the field of forensic science, the phenotypic features of the DNA of the evidence samples have been estimated with certain error rates. In recent years, the estimation and analysis of new multiple CpG regions with a linear correlation between DNA methylation levels and biological age for forensic age estimation and error rates in age estimates tend to decrease. The aim of this study is to reveal the recent developments in this field and to evaluate the use of epigenetic-based age estimates for forensic purposes.

2. DNA Methylation

DNA methylation occurring in the 5' position of cytosine in CpG dinucleotide is a genetically programmed type of DNA modification in mammals (Kohli et al., 2013). DNA methylation analysis has proven to be a reliable method in estimating age in forensic sciences (Vidaki et al., 2013; Yi et al., 2014; Yi et al., 2015; Zbiec-Piekearska et al., 2015) since the relationship of DNA methylation with age has been proven (Hernandez et al., 2011; Johansson et al., 2013; Steegenga et al., 2014).

2.1. *DNA Methylation and Environment*

Studies in identical twins have focused on identifying links between environmental or aging and long-term epigenetic effects on the phenotype. Recent studies support the hypothesis that DNA methylation

acts as a cross between the constant genome and the dynamic environment. In contrast to changes in DNA methylation patterns, environmental stress during the first years of life or throughout life serves as a lifelong genome adaption mechanism and activates the same genome to express different phenotypes (Szyf, 2011). Many studies examining identical twins have determined the relationship between environmental and aging by means of phenotypic persistent epigenetic effects (Fraga et al., 2005; Wong et al., 2005).

Because DNA base sequences are the same, the investigation of identical twins for forensic case solutions is one of the most troublesome subjects of forensic sciences. Because identical twins share the same genetic basis serves as an ideal system for epigenetic research. In a study that examined DNA methylation levels in monozygotic twins, while there was almost no difference between twins in the early stages of life, there were significant differences between twins over 28 years of age (Fraga et al., 2005). Scientists thought it could be explained in a wide spectrum on non-genetic age-related differences on 'gene traces', anthropomorphic characteristics, usually observed in identical twins or disease predisposition (Fraga et al., 2005).

2.2. *DNA Methylation as a Biological Indicator*

Genes that show high levels of DNA methylation in regulatory regions are usually not expressed in the transcription phase and DNA methylation accumulates in the long term leading to silencing of the genes. Epigenetic inaccurate programming, which leads to abnormal DNA methylation patterns (hyper/hypo-methylation) is significantly observed in many diseases and malignant tumors. Hypermethylation generally occurs in CpG regions in promoter regions of cancer-specific genes and is associated with gene inactivation. Hypomethylation throughout the genome is associated with the progression of cancer through different mechanisms (Wild et al., 2010). Interestingly there is evidence supporting two different hypotheses about how hypomethylation occurs. One involves 'passive' loss of DNA methylation in cell division and the other involves the 'active' and potentially much faster loss of methylation independent of DNA replication.

2.3. *DNA Methylation Analysis*

There are many techniques to measure the level of DNA methylation (Kristensen et al., 2009); but most of these techniques require a large amount of DNA or a control experiment. However, in recent years the developed method of pyrosequencing provides the opportunity to work with less biological samples and is relatively suitable for use in forensic laboratories (Park et al., 2014; Zbiec-Piekarska et al., 2015). In addition, this technique is considered to be the most reliable method to determine the level of DNA methylation (Tost et al., 2007). Until today, more than 1000 Human Methylation 450 Bead Chip data sets are stored in the National Center for Biotechnology Information Gene Expression Omnibus-GEO in the USA. Detection of DNA methylation patterns in a single CpG region, gene, or whole methyloma has recently become a growing value, particularly in the field of medical diagnosis. Recommended methods for methylation detection:

a) Chemical modification of unmethylated cytosine residues

b) Protein interaction with 5-methyl cytosine

c) Methylation sensitive restriction enzymes

However, in some cases a combination of techniques may be used, depending on the scientific purpose (Ammerpohl et al., 2009).

2.4. *Forensic Science and Age Estimation by DNA Methylation Levels*

The relationship between the levels of DNA methylation and age, which is the subject of this review, has been shown in studies conducted since 2000s. Along with aging, both increases and decreases in DNA methylation levels occur depending on the tissue and gene (Richardson et al., 2003). These changes lead to the development of malignancy with aging, and may also have pathological consequences that contribute to other diseases (Richardson et al., 2003). Considering the global genome, it is observed that DNA methylation levels mostly decrease with aging (Gentilini et al., 2013; Peng et al., 2012). However, DNA methylation levels have been reported to increase with age in some specific CpG regions (Beerman et al., 2013; Samuel et al., 2012).

In forensic sciences, determining the age of individuals in some cases is an important problem. When a skeleton is uncovered, age estimation (biological age) of the dead can be done by examining various morphological changes related to age in bones or teeth (Lynnerup et al., 2010). These methods produce a relative estimation with a wide variety. Moreover, it is not possible to use these methods on biological samples obtained from the scene and without skeleton or skeleton fragments.

A number of changes in the natural process tissues and organs, which cause aging, can be examined at the molecular level. So far, several methods have been developed for the aging process except from the DNA methylation studies at the molecular level. But despite all the efforts of scientists, all these proposed methods have limitations and causes loss of material in most cases and exhibit low accuracy (Meissner et al., 2010). Studies by Zubakov et al. based on the rearrangement of DNA T-cells in blood have attracted attention as a reliable age estimation method (Zubakov et al., 2010). However, researches have reported error rates of ±8.9 years. Therefore, this method is more suitable for use only in recommended age groups, rather than full age. It is also necessary to pay attention to the diversity of gender and population groups in the implementation of the method. And people with pathological blood disease are not suitable for this template.

Epigenetic analysis, DNA methylation, which is one of the mechanisms of cell differentiation and aging, may serve as age prediction/detection method. Cells and tissues differentiate during growth and this process may involve changes in gene expression and DNA mutation. Considering that the identical twins start with almost the same methylation patterns, it is seen as an ideal model for age-related DNA methylation differences (Bocklandt et al., 2011). There are many studies examining epigenetic status of identical twins in aging studies (Li et al., 2011; Sahin et al 2011).

Bocklandt et al. used genomic size methylation analysis from the saliva sample of 34 pairs of identical twins (21-55 years old) using Illumina Human Methylation microarrays. This indication was not duplicated after advanced statistical analysis in Bocklandt's study. But a subset of 88 new loci was highly correlated with the age detected (Bocklandt et al., 2011). Based on 3 CpG regions (NPTX2, EDARADD, TOM1L1), a regression model was created with average ±5.2 accuracy for the person's age estimate.

Koch and Wagner analyzed several data sets from 13 different cell types or tissues from local data stores (Koch et al., 2011). First, they identified age-related hypermethylated 431 and age-related hypomethylated 25 CpG regions. Then, they chose a subset from the 5 CpG regions to integrate into the epigenetic aging mark (TRIM58, KCNQ1DN, NPTX2, BIRC4BP and GRIA2). Especially, one of these CpG regions (NPTX2) was used by Bocklandt et al. in their own work. Based on the selected CpG region, estimates can be made with average ±9.3 year accuracy (Bocklandt et al., 2011).

Bekaert et al. selected 4 genes (ASPA, PDE4C, ELOVL2, and EDARADD) related to age and identified CpG methylation levels on 206 blood samples from living and dead people (age range: 0-91). They investigated the estimation accuracy of this data with linear and non-linear regression models (Bekaert et al., 2015). They achieved high levels of accuracy with ELOVL2 methylation levels in the quadratic regression model. They found an average of 3.75 years difference between chronological age and predicted age and R^2=0.95 correlation. No difference for the samples obtained between two genders in terms of accuracy. There were no differences in the accuracy rate of samples from people living and dead in both sexes. Researchers also published estimation accuracy of their results according to age groups (Table 1) (Bekaert et al., 2015).

Table 1. Number and percentage of accurate and inaccurate age estimates

Age	0-19	20-39	40-59	60-91	Total
Total inaccurate	1 (3.6%)*	4 (6.6%)	14 (21.9%)	23 (45.1%)	42 (20.4%)
Total accurate	27 (96.4%)	57 (93.4%)	50 (78.1%)	28 (54.9%)	162 (79.6%)
Total	28	61	64	51	204

*Estimated and chronological age was considered correct prediction when matched as ±5 years.

Weidner et al. used Human Methylation 450 Bead Chip technique to identify age-related DNA methylation markers in blood. In this study, pyrosequence analysis based on the combination of 3 DNA methylation markers yielded ±5 years of accuracy in estimating age (Weidner et al., 2014).

In forensic areas, the ELOVL2 promoter is considered the most promising locus for age prediction (Garagnani et al., 2012; Florath et al., 2014; Johansson et al., 2013). Yi et al. found an average of 4 years between predicted and actual age with estimating using multiple linear regression models on blood samples (Yi et al., 2014). Likewise, Zbiec-Piekarska et al. used a multiple linear regression model based on the simultaneous analysis of the 5 CpG regions they tested in the blood and found an error rate of ±3.9 years (Zbiec-Piekarska et al., 2015).

Horvath, a German scientist who has extensive work on DNA methylation and age, has created a large database on the computer to allow online age estimation (Horvath, 2013).

Andrew et al. studied the mtDNA control region and reported no significant difference between genders on the accuracy of age estimates based on DNA methylation analysis (Andrew et al., 2011). Bakeart et al. studied 4 age-associated genes (ASPA, PDE4C, ELOVL2, and EDARADD). Similarly, Bakeart et al. reported in their study, an average of 3.53 years for men, and an average age of 3.95 years for women and there is no statistical difference between estimates for both genders (Bakeart et al., 2015).

Li et al. have formed DNA methylation algorithm for age estimation. They stated that it is not possible to clarify the complex relationship between DNA methylation and age using a simple linear model. They used the Gradient Enhancer Regressor (GER) model to minimize the estimation error and increase the accuracy of the model. It is also examined the methylation data of 278 saliva samples to test their strength when selected age-dependent CpG regions were applied to non-blood body fluids. And it is stated that they found the correlation coefficient as 0.85 between the predicted age and the actual age (Li et al., 2018).

The most widely used DNAm microarray, Illumina Infinium Human Methylation 450 (450K sequence), has recently been replaced with the Illumina Infinium Human Methylation EPIC (EPIC sequence). Thus, the number of targeted CpG fields nearly doubled. Mc Even et al. Infinium Methylation provided support for the use of age acceleration residual metric measure in their work using EPIC Bead Chip technology (Mc Even et al., 2018).

Conclusion

In recent years, the importance of biological samples obtained from the scene increased with increasing crime incidents and the introduction of new techniques. It is not easy to make a simple and precise estimation of the molecular level from biological materials, because the aging process is quite biologically complex. However, current and future research associated with age-related epigenetic patterns may have the potential to change our understanding of aging not only in health but also in disease. Studies have shown that DNA methylation assays are a reliable and effective method for the estimation of forensic age on postmortem and anthropological samples and for the differentiation of identical twins. In addition, it can be used for age estimation in some forensic cases, especially those without material integrity, especially for the crime scene analysis (age estimation of culprit/victim). However, although significant results have been obtained in studies conducted so far, further studies are needed on population and tissue-specific DNA methylation characteristics.

Conflict of interest

The authors declare that they have no conflict of interest.

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Review

Restoration of Endodontically Treated Teeth: A Review of Direct Restorative Approach

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Abstract: Many studies have been conducted on the restorative treatment of endodontically treated teeth, but there is still no consensus. At the same time, restorations of endodontically treated teeth can be very challenging. This article focuses on the characterization of endodontically treated teeth, their pre-restorative assessments and approaches for making more successful restorations with current novel direct restorative materials.

Keywords: *Endodontically treated teeth; restorative treatment; resin composites; cuspal coverage; tooth fracture; adhesive dentistry*

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1. Introduction

Microbial contamination of the root canal system and periapical tissues is the most common reason of the failure in endodontics (Saunders and Saunders, 1994; Torabinejad et al., 1990). Therefore, the root canal system should be sealed both apically and laterally appropriate root filling material in order to prevent microorganisms from reaching the root canal system. Leakage of microorganisms and tissue fluids into the root canal system can occur both apically and coronally. According to the hollow-tube theory (Rickert and Dixon, 1931), it is reported that the toxins formed as a result of the stagnation of tissue fluids at the root ends and the degradation of these fluids maintain the periapical lesion (Wu and Wesselink, 1993). Therefore, many researchers have dipped tooth roots into dyes and scored leakage from the apical to the coronal to detect apical leakage. On the other hand, some studies have reported that sterile tissue fluids cannot cause long-term inflammation, but the inflammation is associated with bacteria and their metabolic byproducts (Makkes et al., 1977; Sundqvist, 1976; Torneck, 1966). It was first reported in 1961 by Marshall and Messler that bacteria and nutrients can also reach the root canal system by coronal leakage (Marshall and Messler, 1961). In 1990, Torabinejad et al. observed bacterial products in the apex of endodontically treated teeth (ETT) without a coronal restoration after 3 months of *in vitro* storage (Torabinejad et al.,

1990). Later, Ray and Trope (Later et al.,1995) conducted a very important study about the role of coronal restoration in the success of ETT. According to this retrospective study, prognosis of ETT was strongly related to the success of coronal restoration rather than the root canal treatment. This study suggests that coronal microleakage is more important than thought, contrary to common belief in endodontics.

Nowadays, it is widely accepted that the prognosis of ETT depends not only on the success of root canal treatment, but also on the success of coronal restoration. In clinical practice, the restoration of ETT is a treatment requiring complicated restorative planning. These treatments can be performed by using indirect restorative techniques or by direct restorative techniques. No matter which technique is chosen, it is known that ETT are weak and more prone to the fracture than vital teeth due to changing in the mechanical properties of dentin (Soares et al., 2007), changing in moisture content (Papa et al., 1994), and reduced levels of proprioception (Randow and Glantz, 1986). However, there are also studies advocating that ETT are not different from vital teeth in terms of fracture strength (Carvalho et al., 2018; Faria et al., 2011; Lewinstein and Grajower, 1981).

Vital teeth are generally fractured as a result of traumas caused by external impacts such as sports, falls, traffic accidents and violence (Goyal et al., 2017). However, ETT can also be fractured under the influence of occlusal function (masticatory forces). Studies have shown that ETT are more susceptible to fraction than vital teeth (González-López et al., 2006; Oskoee et al., 2009). The main reason for the increase in brittleness is the reduced coronal and radicular tissue during the caries removal (Reeh et al., 1989), previous restorations (Lin et al., 2001), intra-radicular procedures (Rao et al., 2013), preparation of the endodontic access cavity (Pantvisai and Messer, 1995; Reeh et al., 1989), and restorative procedures requiring extensive tissue removal (Mondelli et al., 1998; Pantvisai and Messer, 1995). Furthermore, an occlusal cavity preparation has been reported to adversely affect the fracture strength of the tooth between 14 to 44%, while the mesio-occluso-distal (MOD) cavity preparation decrease the fracture strength of 20 to 63% (Larson et al., 1981). The removal of marginal ridges, especially in the occlusal region during preparation, adversely affects the fracture resistance of ETT (González-López et al., 2006). In addition to this, dehydration of the remaining dentin tissue after endodontic treatment and the loss of collagen cross-links have been reported to adversely affect the fracture resistance (Oskoee et al., 2009). Therefore, it is beneficial to avoid unnecessary endodontic procedures and coronal tissue removal that violate the biomechanical balance and compromise the long-term performance of ETT (Magne and Belser, 2002).

2. Functional requirements

Studies have reported that especially teeth with narrow root structure are more prone to fracture as a result of masticatory forces (Chan et al., 1999; Tamse et al., 1998). In particular, maxillary premolar teeth are therefore more frequently fractured (Tamse et al., 1998). Chen et al. found that canine teeth are the most resistant to fractures and reported that incisors tend to fracture only after endodontic treatment (Chen et al.,1999). The force was faced by the anterior and posterior teeth is different from each other. The anterior teeth are mainly exposed to shear and laterally forces, while the posterior teeth are exposed to vertical forces. This difference also affects treatment planning depending on the function. In addition, it was determined that mandibular first molar teeth exhibited two times more fractures compared to

mandibular second molar, maxillary first molar, maxillary second molar, and maxillary second premolar teeth (Chan et al., 1999).

Successful prognosis of ETT correlates with the preservation of dental tissues. Studies have shown that the longevity of the tooth will be prolonged with the conservation of healthier dental tissues (Nagasiri and Chitmongkolsuk, 2005). Costa et al. was supported this idea through their work (Costa et al., 1997). It was found that as the cavity width increased in premolar teeth prepared with MOD cavity, the fracture resistance of the tooth decreased. They also found that the fracture resistance improved significantly when the restorations were completed as onlay, including cusp of the teeth to the preparation (cuspal coverage). In a different *in vitro* study, maxillary premolars presenting only endodontic access cavity preparation were exhibited significantly greater fracture resistance compared to the MOD cavity prepared ones (Steele and Johnson, 1999).

2.1. *Treatment Planning*

Considering the increase in cuspal deflection during function after loss of dental material due to caries removal and endodontic access cavity preparation, and thus becoming more vulnerable to fractures, the question of how these teeth would be better restored may be raised. Although extensive research has been done on the restoration of such teeth, there is still no consensus. In a study about the difficulty in the treatment planning of ETT, different responses were obtained when four different specialists were asked about the treatment planning of the fracture lateral incisor (Türp et al., 2007). It is crucial in treatment planning to answer different questions, such as whether to restore the tooth by direct or indirect technique, whether to use a post or which material is preferred. Therefore, the amount of remaining dental hard tissues and the functional requirements of the tooth should be well evaluated.

2.2. *Preservation of the Coronal Tissues*

The replacement of defective restorations results in larger restorations. This phenomenon was also described by Elderton as the restoration cycle of death (Elderton, 1988). Replaced restorations may then fail again and result in loss of the tooth by performing even larger restorations or post-core restorations. Moreover, Dietschi et al. reported that cavity depth and isthmus width are major factors in determining the stiffness and fracture risk of ETT (Dietschi et al., 2007). With minimal intervention dentistry concept, preservation of dental tissues is gaining importance in the restoration of ETT (Magne et al., 2016, 2017; Yuan et al., 2016).

These treatments can be done with or without intra-canal post systems and cuspal coverage procedures. After final restoration, tooth fractures may occur due to dentinal tissue loss. For this reason, it is recommended to perform intra-coronal reinforcement to prevent tooth fractures (Ayna et al., 2010; Belli et al., 2015).

2.3. *Cuspal Coverage*

After endodontic treatment, restorations with cuspal coverage is a method used to increase the fracture resistance of teeth by reducing the stress formation. The cuspal coverage is simply the removal of the

cusp tips of the tooth after the endodontic treatment to include them within the restoration limits. This procedure can be applied only to functional cusps as well as to all of them (ElAyouti et al., 2011). Many studies have evaluated the effect of cuspal coverage on fracture resistance after endodontic treatment (Bitter et al., 2010; Jiang et al., 2010; Mondelli et al., 2009; Shafiei et al., 2011).

In a previous study, the effects of cuspal coverage on fracture resistance of premolar teeth were investigated. The researchers were reported that 2 mm of reduction in cusps was significantly increased fracture resistance compared to the standard MOD cavity preparation (Mondelli et al., 2009). In a more recently published study, investigators were found that teeth with 2.5 mm of cusp reduction were significantly exhibited higher fracture resistance, and remaining dentinal wall thickness had no role in this improvement (Mishra et al., 2017).

Several studies argued that cuspal coverage along with composite resins enhances the prognosis and minimizes fracture risk (Mondelli et al., 2009; Soares et al., 2008; Torabzadeh et al., 2013; Xie et al., 2012). In normal occlusion, however, some studies reported that cuspal coverage along with the proper adhesive material (Krejci et al., 2003; Mohammadi et al., 2009; Scotti et al., 2011) or if a fiber post is employed (Mohammadi et al., 2009) is unnecessary.

Many studies in the literature have reported that cuspal coverage results in better survival rates (Abu-Awwad, 2019; Aquilino and Caplan, 2002; Sorensen and Martinoff, 1984). However, these studies generally did not consider the amount of dental substance loss. ETT with a MOD cavity and ETT with an occlusal cavity would not have the same risk of fracture (Reeh et al., 1989). Therefore, applying cuspal coverage to ETT in both cases would not comply to the minimally invasive treatment concept. Therefore, overtreatment should be avoided when treating ETTs (Larson et al., 1981; Mannocci et al., 2002; Mondelli et al., 1980).

2.4. *Post Systems*

The posts are the materials used for the formation of the new coronal structure in excessive crown damage with the support of the root canal system after endodontic treatment. Nowadays, the posts are chosen to strengthen the coronal structure in direct restoration after endodontic treatment (Bitter et al., 2010; Mohammadi et al., 2009; Scotti et al., 2011). In the studies evaluating the posts for strengthening the coronal structure after endodontic treatment generally combines cuspal coverage procedure (Mohammadi et al., 2009; Scotti et al., 2011).

Studies have shown that extensively damaged teeth treated using posts are fractured in a way that can be re-treated compared to the treatments without using posts (Mohammadi et al., 2009; Scotti et al., 2011). Fractures are usually more dramatic and result in loss of teeth when the posts are not in use. In a study, no significant difference was observed between post application, cuspal coverage and the combination of these two. In addition, compared to the control group (standard MOD cavity preparation), all three methods significantly increased the fracture resistance (Mohammadi et al., 2009). In another *in vitro* study, which is investigating the influence of the post length, it was reported that the post lengths did not affect the fracture type of the restored teeth, but a significant increase in fracture strength was observed for the use of both post lengths (Scotti et al., 2011).

Generally, posterior ETTs do not require post placement for retention of core build-up restorations in the presence of sufficient intact tissue (Suksaphar et al., 2017). Cagidiaco et al. (2007) and Mannocci et al. (2002) reported 100% survival rate against fracture with the fiber post placement. Dammascke et al. (2013) stated that the fracture rate in direct composite restorations lowers with the post placement. Scotti et al. (2015) also reported that the fiber post application significantly improved the clinical outcome. There are also laboratory studies supporting these findings (Nam et al., 2010). From these findings, post placement in posterior ETTs with excessive substance loss may be beneficial.

2.5. *Fiber Splints*

Fibers are light permeable, aesthetic and easy to apply materials that generally made of polyethylene fiber and glass fiber. Since they are biocompatible, they can be used safely. Fibers are used for splinting of teeth in periodontal and orthodontic treatments, strengthening of direct composite restorations, adhesive bridges in the absence of a single tooth, reinforcing the bases of removable prostheses (Belli et al., 2006; Vallittu, 2018).

After the endodontic treatment, fibers can be used in the restoration of the teeth with composite resin to strengthen the coronal structure. In direct composite restorations, the fiber splint is placed inside the restoration or the cusps are splinted together by the fiber strip (Akman et al., 2011; Manzoor et al., 2018; Oskoee et al., 2009; Tayab and Shetty, 2015; Vallittu, 2018).

2.6. *Restorative Material Choice*

Fractured posterior tooth is a most common clinical problem in restorative dentistry. Fracture resistance of restored teeth depends on the type of restorative material used, the anatomy of the tooth, the position of the tooth in the occlusion, the size of the cavity preparation and the width of the isthmus (Trope et al., 1985). Therefore, if there is no dentine support underneath the cusp, onlay restorations should be preferred. Cuspal coverage of the working cusp should be considered to distribute occlusal forces and to improve bond strength (Christensen, 2012). Dental fracture in restored posterior teeth represents a common clinical problem (Omer et al., 2019). The cusp height should be reduced by cuspal coverage to eliminate higher stress over teeth. However, direct restorations with cuspal coverage enhances fracture resistance against compressive forces (Lin et al., 2008).

2.7. *Direct Restorative Materials*

A recent finite element analysis (FEA) study reported that working cusp reduction enhances the biomechanical properties of dental restoration complex, consequently providing better prognosis (Kantardžić et al., 2012). In an *in vitro* study, researchers did not find a significant difference between direct or indirect approaches when restoring ETT with composite resin (Plotino et al., 2008). In a different study, researchers were suggested that the prognosis of direct restoration depends on the material choice (Torabzadeh et al., 2013). Further, in a more recent *in vitro* study, researchers were concluded that cuspal coverage with an amalgam and composite resin combination exhibited no difference (Shafiei et al., 2011). Restorative

materials chosen for the restoration of ETT requires adequate retention and strength to maintain and protect the remaining dental structures against occlusal forces. Different types of direct restorative materials can be selected as final restoration in which include amalgam, glass-ionomer cement, or composite resin to maintain teeth and restore the function.

Amalgam was preferred due to its resistance to masticatory forces in the posterior region. However, amalgam cannot bond to dental tissues and requires additional cavity preparations that weaken dental tissues to provide mechanical retention (Varga et al., 1986). On the other hand, adhesive restorative materials have aesthetic properties and adequately bond to dental hard tissues without excessive cavity preparation (Assif et al., 1993; Baraban, 1972; Cho et al., 1999).

When endodontic success is mentioned, different results are seen in studies comparing amalgam and composite restorations (Shu et al., 2018). The contradictory findings of the studies conducted in different years can be explained by the developments in composite materials and application techniques (Göhring and Peters, 2003). According to a systematic review, the composites still have a lower longevity and a higher risk of secondary caries than amalgams (Moraschini et al., 2015). Considering that the condition in periapical tissues is related to the success of coronal restoration (Göhring and Peters, 2003), improvements in restorative materials will increase the success of endodontic treatment.

Composites and adhesive systems are widely used because of their aesthetic properties, their ability to bond to enamel and dentin, and theoretically increasing the integrity of the dental restoration complex (Ergücü and Türkün, 2007; Schirrmeister et al., 2009). Many studies have compared different restorative materials and their use (Monga et al., 2009; Soares et al., 2008). In an *in vitro* study, it was reported that composite resin restorations applied to MOD cavities prepared for maxillary premolar had no more reinforcing effect than MOD amalgam restorations performed without adhesive application (Stampalia et al., 1986).

Composite resins have been modified many times in order to eliminate the clinically felt deficiencies since the 1960s. Previously, modifications were made on the filler particles of the material to obtain materials with better mechanical properties that possess high fracture resistance and better polishability (Sakaguchi and Powers, 2012). Later on, it was aimed to reduce the polymerization shrinkage, which is seen as the cause of post-operative sensitivity, microleakage and cuspal deflection (Dayangac, 2011). Today, many new restorative materials are made available to dentists in parallel with the development of adhesive technology. As a result of the above-mentioned goals, fiber-reinforced composites, silorane-based composites and bulk-fill composites are in use by dentists.

Resin composite restorations can increase the durability of the remaining dental tissues of ETTs based on the adhesive concept realized by the adhesion of dental hard tissues and restorative material (Dietschi et al., 2011; Mincik et al., 2016). In an *in vitro* study, the fracture strength of ETT, which was restored with resin composite, was found to be similar to that of the intact tooth (Ausiello et al., 1997). According to some retrospective studies, ETTs restored with resin composite showed a higher survival outcome than amalgam-restored ETTs (Hansen, 1988; Mannocci et al., 2005; Nagasiri and Chitmongkolsuk, 2005). However,

long-term degradation of the hybrid layer is still a concern (Hashimoto et al., 2003). This degradation adversely affects the fracture strength of ETTs restored with resin composite in the long-term (Opdam et al., 2014).

2.8. *Fiber-Reinforced Composites*

Based on the idea that a restorative material capable of dissipating or absorbing stress in high stress areas (e.g. posterior region) will protect the tooth tissues (Fráter et al., 2014), various improvements have been made to the inorganic phases of the resin composite materials. As a result of these improvements, seromers obtained by adding ceramic and fiber-reinforced composites obtained by adding fiber were found (Garoushi et al., 2007; Zandinejad et al., 2006).

In simple terms, a composite structure consisting of fibers held together by a resin matrix is called fiber-reinforced composites. All fiber materials are filamentous materials that can be silanized by OH^- ions on their surfaces, thus good adhesion can be achieved with the resin matrix as a result of this silanization. They were first developed in the 1960s to strengthen the methacrylate base of removable protheses. It was found that the material improved on flexural strength, fatigue resistance, elastic modulus and bond strength by adding fibers to the restorative material structure (Zhang and Matinlinna, 2012). Furthermore, it has been reported that the presence of fiber in the composite structure stops the progression of the crack during fracture process (Braga and Ferracane, 2004; van Dijken and Sunnegårdh-Grönberg, 2006; van Heumen et al., 2009; Manhart et al., 2004). Moreover, fiber reinforcement between the restorative material and dentin changes the fracture line, causes repairable fractures, saving the remaining dental tissues (Belli et al., 2005; Belli et al., 2006), and improves the restorability of ETT after failure (Shafiei et al., 2014).

It is thought that the use of a material more similar to dentine tissue to restore missing dental tissues biomimetically would prevent the progression of cracks due to the forces encountered during the function. As a result of this idea, the most recent composite material is everX Posterior (GC Corporation, Tokyo, Japan). This material is a condensable fiber-reinforced composite material produced to mimic the stress absorbing property of dentin and dentinoenamel junction. In addition, this material is designed as a single layer substrate material consisting 7.2% of short fibers by volume and requires the application of a conventional composite resin on top layer (Garoushi et al., 2008; Garoushi et al., 2015).

In an *in vitro* study, direct onlay restorations with conventional composite and fiber-reinforced composites were compared. As a result, the fracture resistance of the fiber-reinforced composites was found to be higher and when applied in combination with the conventional composite, it increased the fracture resistance of the traditional composite (Garoushi et al., 2008). Nevertheless, cuspal coverage with direct composite restorations appears to be a safe in extensive substance loss (Mondelli et al., 2009; Plotino et al., 2008)

2.9. *Silorane-Based Composites*

A monomer called silorane has been developed to reduce polymerization shrinkage in composite resins. Silorane takes its name from the siloxane and oxirane functional groups. While siloxane imparts a high

hydrophobic property to the structure, cycloaliphatic oxirane, a cyclic ether, improves the durability of the material by exhibiting ring polymerization and reducing polymerization shrinkage (Sakaguchi and Powers, 2012). The water absorption and related discoloration of the material are low due to the hydrophobic properties of siloxane (Zimmerli et al., 2010).

Silorane composites exhibit low polymerization shrinkage and high strength compared to methacrylate composite resins (Eick et al., 2002). Nowadays, these materials are not widely used for reasons such as their application with a special adhesive system and their limited indication for only posterior teeth due to low color choices. There are many studies in the literature on silorane-based composites. A systematic review of these studies concluded that silorane-based composite resins did not show a significant superiority compared to methacrylate-based composite resins and should have long-term clinical follow-up (Maghaireh et al., 2017). Moreover, silorane-based composite resins showed similar clinical performance as conventional composites (Magno et al., 2016). Although Lien and Vandewalle (Lien and Vandewalle, 2010) reported the compressive strength and the microhardness of the restorative materials to be low, silorane-based composites markedly increase the fracture resistance of ETT (Shafiei et al., 2014) and decrease cusp fracture in MOD cavities (Palin et al., 2005). Fiber reinforcement had no effect on the fracture resistance of the restoration, whereas the use of a nano-ionomer core under the silorane-based restoration exhibited an improvement in terms of fracture resistance (Shafiei et al., 2014).

2.10. *Bulk-Fill Composites*

One of the recent developments in composite resins is the production and launch of bulk-fill composites to the dental market. Conventional composite resins are introduced into the cavity by the incremental technique, thereby allowing the light used in the polymerization to better penetrate into the material and reduce the polymerization shrinkage stress (El-Safty et al., 2012). The incremental technique has disadvantages such as the presence of air bubbles between the composite layers, inadequate bonding of the two layers, and long operating time (Garapati et al., 2014).

The major advantage of bulk-fill composite resins is that they can be placed in a single increment (bulk) of 4 to 6 mm thickness and exhibit low polymerization shrinkage (El-Damanhoury and Platt, 2014; Monterubbianesi et al., 2016). Other advantages include shorter application time, ease of application, good adaptation of the composite to the cavity, adequate wear resistance to masticatory forces, adequate radiopacity, good polishing and aesthetic properties (El-Damanhoury and Platt, 2014; Monterubbianesi et al. 2016). In another study, in cavities lined with SDR (Dentsply Caulk, Mildford, DE, USA), cuspal deflection is reduced markedly (Moorthy et al., 2012). SDR results in reduced polymerization shrinkage in comparison to Filtek Supreme Flow (3M, St. Paul, MN), Esthet X Flow (Dentsply Caulk, Mildford, DE, USA), nano-hybrid, microhybrid, and silorane-based composites (Ilie and Hickel, 2011).

3. Clinical Considerations

The treatment of ETTs without diffuse destruction is usually performed with direct composites (Baratieri et al., 2000). Some authors suggest that cuspal coverage, direct and indirect restorations show similar clinical

outcome, and therefore direct restorations should be preferred because their cost and time efficiency (Angeletaki et al., 2016; da Veiga et al., 2016; Fennis et al., 2014). On the other hand, the skill level and accuracy of the clinician is of great importance in the application of direct restorations and affects the outcome of restorative treatment (Laske et al., 2016). For example, there are some risks in the direct restorative techniques, such as polymerization shrinkage, technical sensitivity, incompatible proximal contact, micro leakage and secondary caries formation (Alshiddi and Aljinbaz, 2016; Bianchi et al., 2013).

The first factor that should be evaluated in the conservative treatment of ETTs is the present state of the teeth. According to the recently published study, by evaluating the loss of substance, we can simply divide the ETTs into three categories: minimally destructed, moderately destructed, and severely destructed (Abu-Awwad, 2019).

Minimally destructed ETTs are teeth with only endodontic access cavity or where only one of the marginal ridges is missing (MO or DO cavities) with the support of axial walls of sufficient thickness (≥2 mm). There is no need for cuspal coverage in the conservative treatment of ETTs in this category. Nagasiri and Chitmongkolsuk (2005), reported that minimally destructed ETTs had a survival rate of 78% after 5 years of follow-up in their retrospective clinical study. Mannocci et al. (2002) also reported that the premolars with minimally destruction had a high survival rate in 3-year clinical follow-up. Similar supportive findings have been reported in both clinical (2013) and laboratory (Reeh et al., 1989; Steele and Johnson, 1999) studies. Unlike the minimally destructed ETTs, the ETTs are defined as moderately destructed if they do not have axial walls of sufficient thickness (<2 mm) or have lost both of its marginal ridges (MOD cavity). Cuspal coverage results in successful clinical outcomes for this category (Pantvisai and Messer, 1995; Reagan et al., 1989; Reeh et al., 1989; Scotti et al., 2011, 2013; Sorensen and Martinoff, 1984; Steele and Johnson, 1999). Severely destructed ETTs are cases where there is more substance loss than the MOD cavity. These ETTs would benefit from the cuspal coverage procedure; besides intraradicular retention should be considered (Afrashtehfar et al., 2017).

On the other hand, failure of restorative treatment may be influenced by localization of ETT in occlusion. In a long-term clinical study, mandibular premolar and anterior teeth both in maxillary and mandibulary have been reported to have longer survival outcomes (Cheung and Chan, 2003). In the same study, it was reported that molar teeth had lower survival rates. In another study on direct restorations, it was reported that molar teeth (5.2%) have a higher annual failure rate than premolar (4.0%) and anterior teeth (4.4%) (Laske et al., 2016).

Another controversial issue on the treatment of ETT is the use of posts. *In vitro* studies have shown that the use of fiber post improves the fracture strength of ETT (Abduljawad et al., 2016). Furthermore, in a clinical study, the survival rate of post-treated teeth (94.3%) was significantly higher than that of unused teeth (76.3%) (Guldener et al., 2017). In spite of this, some authors state that preparing a post space may increase the risk of root fracture (Faria et al., 2011; Göhring and Peters, 2003). In addition, Belleflamme et al. (2017), concluded in their 10-year retrospective study that practitioners should consider the endocrowns instead of the post and core approach to restore severely destructed ETTs. Therefore, it would be appropriate to avoid post use except severely destructed ETT, parafunction or excessive lateral forces.

Conclusion

It has been demonstrated in many scientific studies that ETT are more prone to fracture than vital teeth. This fact should not be ignored in the choice of restorative approach and material. Direct restorative approaches can be applied safely for the teeth that do not show excessive substance loss after endodontic treatment.

It has been shown that the ideal stress distribution is achieved by using materials that can be attached to dental tissues in the direct restorations to be applied after endodontic treatment. In addition, it is reported that the modulus of elasticity of the restorative material to be used should be close to the dental tissues in order to reduce the amount of stress due to masticatory forces for the remaining dental tissues. In the light of this information, it could be concluded that the most ideal restorative material to be applied after endodontic treatment would be composite resins, in particular fiber-reinforced composites.

It is observed that cuspal coverage, post system applications and fiber splint applications increase the fracture resistance of ETT and provide more ideal stress distribution. It has also been reported that fractured tooth tissues can be restored if fiber post systems or fiber-reinforced composites were used. In conclusion, the prognosis of ETT would be increased when the intra-coronal reinforcement done.

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Conflict of Interests

Author declares no conflict of interests.

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Review

Medication Related Osteonecrosis of the Jaws

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Abstract: Medication-related osteonecrosis of the jaw (MRONJ) is a severe adverse drug reaction, including of bone destruction in the jaws. Osteonecrosis of the jaws can be caused by two pharmacological agents: antiresorptive (including bisphosphonates (BPs) and receptor activator of nuclear factor kappa-B ligand [RANK-L] inhibitors) and antiangiogenic. Among the drugs associated with the development of MRONJ, BPs are the most widely used for a wide variety of clinical indications. Bisphosphonates are used in bone metabolism-related diseases such as Paget's disease, fibrous dysplasia, osteogenesis imperfecta, but mainly in lung, prostate, breast carcinomas, multiple myeloma and osteoporosis. The effect of the drug depends on the dose and duration of administration. Epidemiological studies showed that long-term use of these drugs may increase the risk of MRONJ development. The purpose of this review is to define the current information about MRONJ, the management strategies and preventive measures.

Keywords: *Bisphosphonate; drug therapy; osteochemonecrosis; osteonecrosis of the jaw*

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1. Introduction

Medication-related osteonecrosis of the jaw (MRONJ) can occur after exposure to antiresorptive agents including bisphosphonates or denosumab, or angiogenesis inhibitors to avoid bone complications (Khan et al., 2015).

MRONJ is more common among patients receiving high cumulative doses of bisphosphonates or denosumab than in patients receiving lower doses (de Boissieu et al., 2017; Khan et al., 2017). MRONJ was first reported in 2003 by Marx who reported 36 patients have a history of treatment with bisphosphonates and painful bone exposure in the mandible and maxilla (Marx, 2003). After establishing an relationship

between MRONJ and bisphosphonate treatment, cases related to denosumab treatment began to emerge in 2010 (Aghaloo et al., 2010; Taylor et al., 2010).

The pathogenesis of MRONJ may be multifactorial and have an association between local trauma/infection and reduced bone turnover after exposure to antiresorptive agents (Nicolatou-Galitis et al., 2019).

Furthermore MRONJ is also related with anticancer agents, including angiogenesis inhibitors, classic chemotherapy agents, tyrosine kinase inhibitors (TKIs), immunotherapeutic agents, and inhibitors of mammalian target of rapamycin (Nicolatou-Galitis et al., 2019).

The purpose of this review is to define the current information about MRONJ, the management strategies and preventive measures.

2. Molecular Mechanisms Regarding Osteochemonecrosis

The anticancer agents used in conventional treatment are not selective for tumor cells and other tissues (Greish, 2007). Therefore, all chemotherapeutic agents have side effects on normal tissues and organs (Leaf, 2004). To understand MRONJ physiopathology, the molecular mechanisms that are responsible for bone formation and remodeling in this process should be known.

The capillaries consisting of arterioles and venules have a regular and functional structure (Jain, 2003). Tumor vessels are generally composed of endothelial cells that are unordered, damaged and have wide lumens and in particular have impaired receptor function against angiotensin II, vasoactive mediators (Hashizume et al., 2000; Yuan et al., 1995). In addition, because of increased production of vascular permeability enhancing factors such as bradykinin, nitric oxide, vascular growth factors and prostaglandins, the transition of macromolecular drugs to tumor tissue is easier compared to normal tissues (Eskiizmir et al., 2017; Wu et al., 1998).

All these factors increase the passage through the tumor capillary and cause selective delivery of nano-sized macromolecular drugs to tumor tissues (Alberto et al., 2001; Kopecek et al., 2001). In addition, low lymphatic fluid flow in tumor tissue increases prolonged effect of the drug in tumor tissue. This effect is called enhanced permeability and retention effect (EPR) (Matsumura and Maeda, 1986).

Because of the abnormal structure and function of blood and lymph vessels around the tumor, an increase in fluid pressure between tissues is observed (Fukumura and Jain, 2007). As the selectivity of the tumor vessels decreases, permeability increases and hypoxia and acidosis occur in the tumors due to their abnormal metabolic environment (Harris, 2002; Tatum et al., 2006). Hypovascular regions are formed within the tissue due to unstable development of vessels and excessive proliferation of tumor cells. Chronic hypoxia is also seen in areas that are far from the blood vessels. Because of the lactic and carbonic acids formed as a result of anaerobic glycolysis, pH of the tumor environment is very low (Fukumura and Jain, 2007). Oxygen pressure and pH values affect tumor growth, metabolism and responses to various treatments. These two properties increase the effect of angiogenic factors and cause the tumor growth and metastasis (Fukumura and Jain, 2007).

The main purpose of cancer treatment is to destroy the cancer cell without affecting normal tissues. This can only be achieved by selectively targeting the cancer cell. The features in tumor targeting; increasing the localization of the drug in the tumor by active or passive targeting, decreasing the localization in untargeted cells, minimizing drug leakage from the transition regions, preserving the drug from disintegration, keeping the drug in the targeted area for the certain period of time, facilitating the intracellular uptake and biocompatible and biodegradable of the delivery system components (Lammers et al., 2008).

The conventional, biotechnological and gene-based drugs can be selectively transported to specific areas of the body such as organs, tissues and cells by targeting drugs (Erdoğan M. A, 2019). Passive targeting is the natural targeting of the carrier after intravenous injection. Active targeting is magnetic targeting, ultrasonic targeting, and ligand-receptor mediated targeting (Kaş and Eldem, 2002).

Oncogenic mutations targeting signaling pathways and signaling proteins that control the proliferation and/or survival functions in normal cells are common. Changes in signal transduction eliminate the control of cell proliferation and/or survival functions. Therefore, the oncogenic signal transduction plays an important role in the invasion/metastasis process on tumor development. Protein kinases enable protein phosphorylation/activation during signal transduction. Protein kinases are divided into two main groups: membrane located and cytoplasmic located. Protein kinases located in the membrane are called receptor tyrosine kinases (RTK). RTKs contain the tyrosine kinase domain, responsible for activation, in the cytoplasmic portions. These receptors become active after binding with growth factors and interact with target proteins in the cytoplasm to perform signal transduction (Doğan and Güç, 2004; Fizazi et al., 2010).

The signal transduction is reversible and RTK mediated transmission is controlled under physiological conditions. In the carcinogenesis process, the continuous and uncontrolled RTK activity is observed. Cytoplasmic protein kinases include tyrosine kinase inhibitor such as Src, Abl, focal adhesion kinase (FAK), janus family kinase (JAK) proteins. Similar to membrane tyrosine kinases, the continuous and uncontrolled activation of cytoplasmic tyrosine kinases and oncogenic signal transduction accelerate malignant properties such as transformation, tumor growth, motility, invasion, and angiogenesis. Mutations leading to continuous activation of protein kinases and over-expression of protein kinases can lead to oncogenic transformation (Doğan and Güç, 2004; Fizazi et al., 2010).

The inhibition of RTK signaling is important in cancer treatment. Two different types of compounds are used to inhibit RTK signaling. The first is tyrosine kinase inhibition (TKI) with small molecular weight agents and the other is monoclonal antibodies (mAb). Although RTK signal is inhibited in both ways, there are also different target epitopes (the region on antigen molecule that bind specific receptors on the antibody or sensitive lymphocyte, which determines the specificity of antibody or lymphocyte) and activation mechanisms. TKIs bind competitively to the ATP binding site in the catalytic part of the receptor and inhibit both autophosphorylation and intracellular signal transduction. These agents can be displaced between plasma membranes and interact with cytoplasmic fragments of cell surface receptors and intracellular signaling molecules. mAbs can only act on the molecules expressed or secreted on the cell surface. They do not have the ability to pass through the cell membrane (Macfarlane and Chi, 2010).

Monoclonal antibodies are the most widely accepted and approved therapy in the cancer immunotherapy methods (Waldmann, 2003). Nowadays, the main antibodies produced as drugs are monoclonal antibodies such as trastuzumab, cetuximab, bevacizumab, alemtuzumab, rituximab. They target mainly breast cancer, colon cancer and various blood cancers. They target cancer-promoting growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), or antigens specifically produced in cancer cells such as CD52 and CD20. Ipilimumab prevents the immune system inhibition by blocking the cytotoxic T lymphocyte antigen -4 (CTLA-4) antigen (Hodi et al., 2010; Hurwitz et al., 2004; Keating et al., 2002; Reff at al, 1994; Waldmann, 2003).

The epidermal growth factor receptor (EGFR) is an overexpressed tyrosine kinase receptor in many types of cancer such as breast, ovary, colorectal and head and neck cancer. It is involved in cancer cell proliferation, tumor growth, angiogenesis and metastasis. Cetuximab is a monoclonal antibody targeting EGF and clinically approved for cancer immunotherapy (Hamid, 2004).

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor and growth factor and belongs to the same family as EGFR. HER-2 is expressed as a growth factor abnormally high in 25-30% of breast cancers. In breast cancer with increased HER-2 expression, the disease is more aggressive and shorter survival is observed in women (Press et al., 1993; Ravdin and Chamness, 1995; Seshadri et al., 1993). Trastuzumab is an IgG1 isotype monoclonal antibody that binds to HER-2. It inhibits the signaling pathways in cancer cells by binding to HER-2 (Slamon et al., 2001).

CD20 is a differentiation antigen found in normal and malignant B cells but not in precursor B cells (Chamuleau et al., 2010). Rituximab is an IgG1 isotype chimeric monoclonal antibody directed against the CD20 antigen. This antibody has been shown to be effective in the treatment of B cell non-Hodgkin lymphomas (Maloney et al., 1997).

CD52 is a differentiation antigen found in all lymphocytes and has been associated with some lymphomas (Buggins et al., 2002; Piccaluga et al., 2007; Ratzinger et al., 2003). Alemtuzumab is an IgG1 isotype monoclonal antibody directed against the CD52 and clinically approved for the treatment of B cell leukemia (Riechmann et al., 1988).

CTLA-4 (cytotoxic T lymphocyte antigen4) is expressed in the membrane of helper T cells and involved in transmitting inhibitory signals to T cells. Therefore, it attempts to prevent the inhibition and suppression of the immune system. It has provided high survival rates in advanced melanoma patients compared to normal treatment with ipilumumab (Beck et al., 2006).

One of the most important factors in the formation of new vessels is VEGF. Serum VEGF levels were increased in the majority of patients with metastatic cancer (Kraft et al., 1999). Bevacizumab is an IgG1 isotype monoclonal antibody that targets VEGF. This antibody inhibits angiogenesis by binding to VEGF (Los et al., 2007). Bevacizumab is approved for clinical use in metastatic colorectal cancer and non-small cell lung cancer, since it significantly improves survival rate when combined with chemotherapy in patients with metastatic colorectal cancer (Hurwitz et al., 2004).

There are more monoclonal antibodies used in the treatment of cancer. There are various side effects such as fatigue, fever, headache and muscle pain, difficulty in breathing, hypertension, hypothyroidism, dry skin and coloration, hair color change, mild erythema and hyperkeratosis, symmetrical painful, erythematous and swollen areas and petechial hemorrhages, hyperglycemia, hyperlipidemia, stomatitis and pneumonia, hepatotoxicity, arrhythmias (Elloumi et al., 2012; Şakalar et al., 2013).

One of the proteins controlling physiological and pathological bone resorption is the receptor activator nuclear kappa B (RANK) that is found in osteoclasts and cause bone destruction by stimulated with RANK ligand (RANKL). Bone mass is determined by the coexistence of osteoblasts and osteoclasts. Denosumab has a negative effect on osteoblast formation and function by decreasing the effect of RANK molecules on osteoclasts in bone metastases of prostate cancer (Demirkazık and Özal, 2010; Henry et al., 2009; Rosella et al., 2016). Unlike bisphosphonates, it is not nephrotoxic, therefore it is thought to be an alternative to zoledronic acid in patients with bone metastases with renal dysfunction. Recent studies of breast and prostate cancer with bone metastases have shown superiority to zoledronic acid in preventing and delaying skeletal-related events. It has also been shown to reduce metastatic bone pain, like zoledronate (Henry et al., 2009). Also the mucosal and bone changes of jaws may be occurred by this group medication, but as an important feature, frequent improvements and frequent recurrences are common (Özbayrak and Pekiner, 2016).

3. Bisphosphonate Related Osteonecrosis of the Jaw

Bisphosphonates are structural anologues of endogenous non-metabolized inorganic pyrophosphonates that regulate the bone mineralization and are drugs that inhibit osteoclastic function. In the healthy bone, osteoblastic bone formation occurs by osteoblast migration to the osteoclastic resorption site (remodeling mechanism). These two functions are essential for the repair of physiological disturbances. Bisphosphonate group drugs easily bind to hydroxyapatite crystals at remodeling sites. They are released from this area and absorbed by osteoclasts. The bisphosphonate inhibits the synthesis of farnesyldiphosphonate, a key enzyme of the mevalonate pathway that produces cholesterol within the cell, so the surface properties required for osteoclast to bone resorption. Therefore, the osteoclastic activity and new bone formation is suppressed due to this. This group of drugs also delays the wound healing and tissue repair by inhibiting vascular formation with its antiangiogenic properties (Dumlu et al., 2011; Pekiner, 2014).

High doses of bisphosphonates are used to prevent tumor invasion by causing cytotoxic effect on osteoblasts, apoptosis of tumoral cells. While the half-life of bisphosphonates in the bloodstream is 30-120 minutes, the unmetabolized bisphosphonate in the bone can remain for up to 10 years. It is known from the *in vitro* studies that bisphosphonates disrupt endothelial cell proliferation, adhesion and migration. When the active substance reaches a sufficient concentration in the bone, they also create toxic effects on the soft tissue covering the bone surface and inhibit the epithelial cell proliferation (Özbayrak and Pekiner, 2016).

The effect of the drug depends on the dose and duration of administration. Since bisphosphonates increase bone mineral density, they are widely used by oral administration for the prophylaxis of bone

fractures related with osteoporosis. 10 mg/day per oral administration increases the probability necrosis within 4 years. Once a month intravenous administration used, the probability of necrosis increases within 2 years (Özbayrak and Pekiner, 2016).

Bisphosphonates are used in bone metabolism-related diseases such as Paget's disease, fibrous dysplasia, osteogenesis imperfecta, ankylosing spondylitis, but mainly in lung, prostate, breast carcinomas, multiple myeloma and other malign tumors (Özbayrak, 2017).

Bisphosphonate used before the eruption period may cause dental retention due to the deterioration of osteoclastic activity in the eruption pathway. Gastrointestinal intolerance and esophageal ulcers may be occured in oral administration of the preparation, influenza-like symptoms such as fever, myalgia, arthralgia, nausea, vomiting, as well as uveitis-scleritis and renal failure may be occured with i.v. administration (Özbayrak and Pekiner, 2016).

Bisphosphonate group drugs are divided into two main subgroups;

Nitrogen-containing bisphosphonates (aminobiphosphonates); there are long R2 chains containing nitrogen. Their effects are 100-2000 times stronger than those that do not contain nitrogen (alendronate, risedronate, pamidronate, ibandronate, zoledronic acid) (Marx, 2011).

Nitrogen-free bisphosphonates; known as first generation bisphosphonates. R2 chains are short. When they reach bone tissue, they are captured by osteoclasts and converted into toxic analogs of adenine triphosphate in the cell and show their effect by this way. They are metabolised very quickly in the body (etidronate, tiludronate, clodronate) (Marx, 2011).

The chemical difference prevents the bisphosphonates from hydrolyzing in acidic environment and allows them to remain in the tissues for longer. The carbon at the center of the bisphosphonates binds to hydroxyapatite in bone, and the peripheral chains (R1, R2) are variable. The major chain responsible for the antiresorptive mechanism is R2 chain, the nitrogen group (Özbayrak and Pekiner, 2016).

Due to the rapid metabolic functioning and high turnover of the jaw bones and the deterioration of this cycle related the effect of the drug, the bone/mucosal necrosis occurs to a large extent in the jaws. The remodeling rate of the alveolar bone is 10 times higher than the femur and tibia. Osteonecrosis is common more frequently in the jaw bones because the jaw bones are frequently exposed trauma such as prosthesis, so the integrity of the fine periosteum and mucosa easily deteriorates and allows microorganisms to settle (Marx, 2011). 1/3 of the cases occur in the maxilla and 2/3 of the cases occur in the mandible. Up to 5% of cases can involve both jaws. In addition, while 25% of the cases occur spontaneously, 75% have related with a dental procedure (Özbayrak and Pekiner, 2016).

A wide variety of potential risk factors for the development of osteonecrosis is divided into two, locally and systemically (Nicolatou-Galitis et al., 2019) (Table 1).

Osteonecrosis lesions are clinically asymptomatic or or in some cases blunt pain but usually severe pain (70%) is observed. Paresthesia may also develop according to localization of lesion (Figure 1-3). In addition,

osteomyelitis, sequestration, trismus, halitosis, extra/intraoral fistula, anesthesia, actinomyces and many other microorganism-related infections, mobility of adjacent teeth may be detected (Font et al., 2008).

Table 1. Risk factors for the development of osteonecrosis of the jaw

Local risk factors	**Systemic risk factors**
Tooth extraction	Use of corticosteroids
Surgical procedures	Immunosuppressive therapy
Subgingival curettage	Anemia
ill-fitting dentures	Diabetes mellitus
Dental / periodontal infection	Smoking
Irritation of the apical periodontium	
Poor oral hygiene	

Figure 1. (a) Clinical and radiological view of bone necrosis around the teeth 27 after 10 months administration of i.v zoledronic acid in breast cancer (b) Diffuse bone necrosis in the clinical and radiological image of the same patient resembling periodontal abscess in teeth region 34-35.

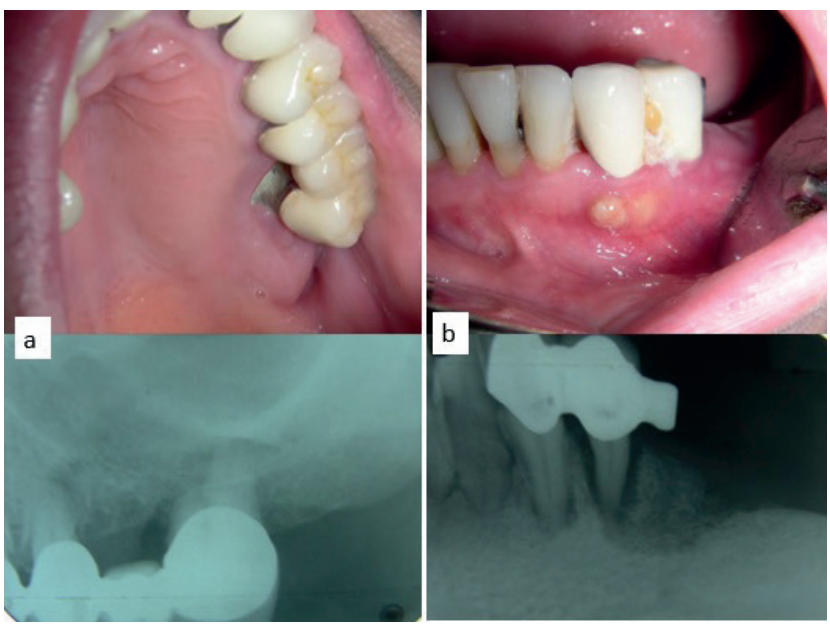


Figure 2. The oro-nasal communication with perforation of the soft tissue and large necrotic area of the palatinal bone in a patient using zoledronic acid for breast cancer.

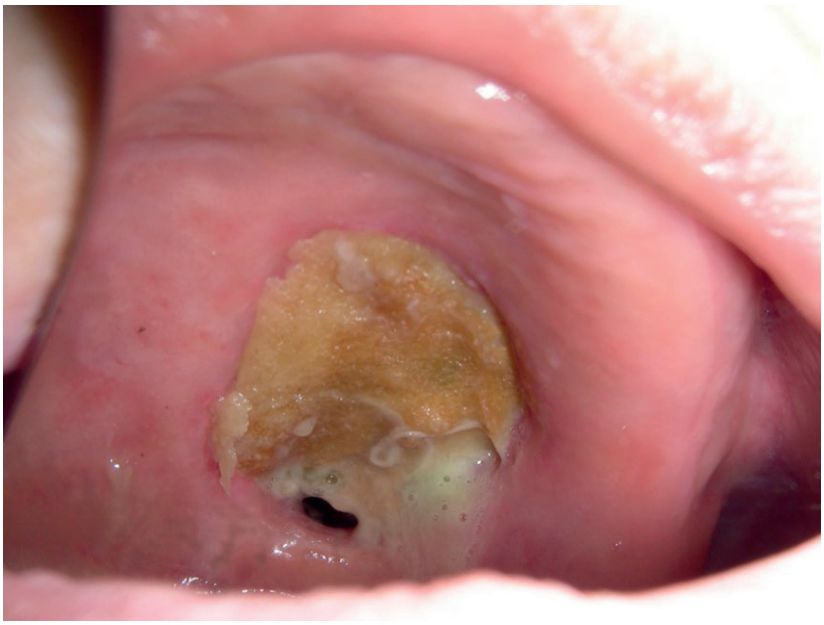
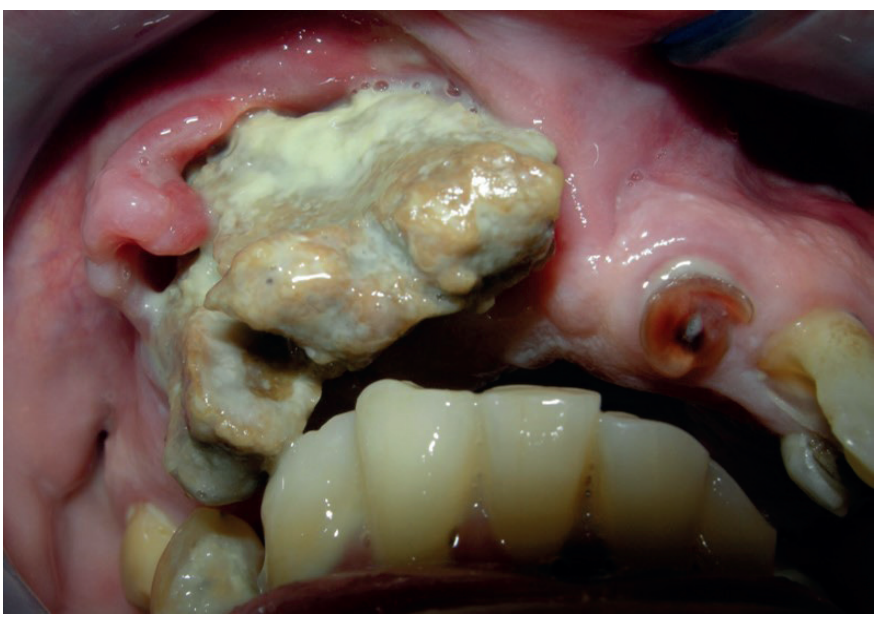


Figure 3. The large sequester due to i.v. zoledronic acid administration once a month for 2 years in the breast cancer.



Radiological findings are not initially specific. Initially, the periodontal ligament space widening, as the lesion progresses large radiolucent areas, spontaneous fracture, increase in bone mineral density, change in bone formation, erosion in cortical bone, new bone formation in periostal bone and sequester may be observed (Özbayrak and Pekiner, 2016) (Figure 4 and 5).

Figure 4. The necrotic bone due to zoledronic acid administration in the multiple myelom and the sequester separated clearly from intact bone on OPTG and axial CT scan.

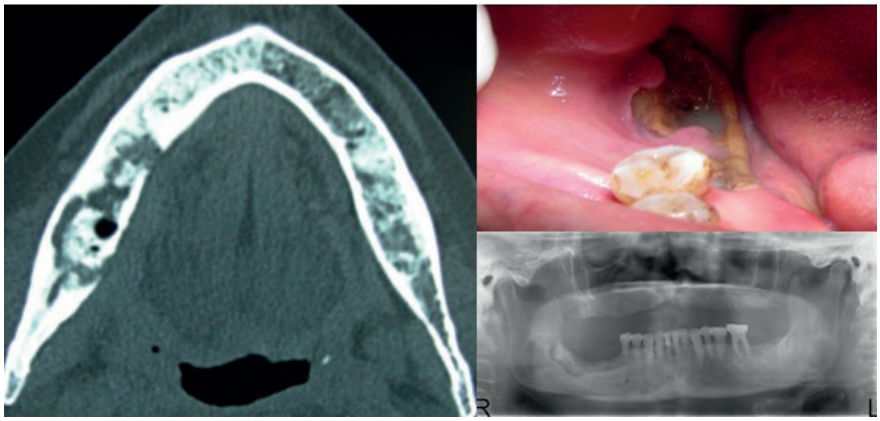
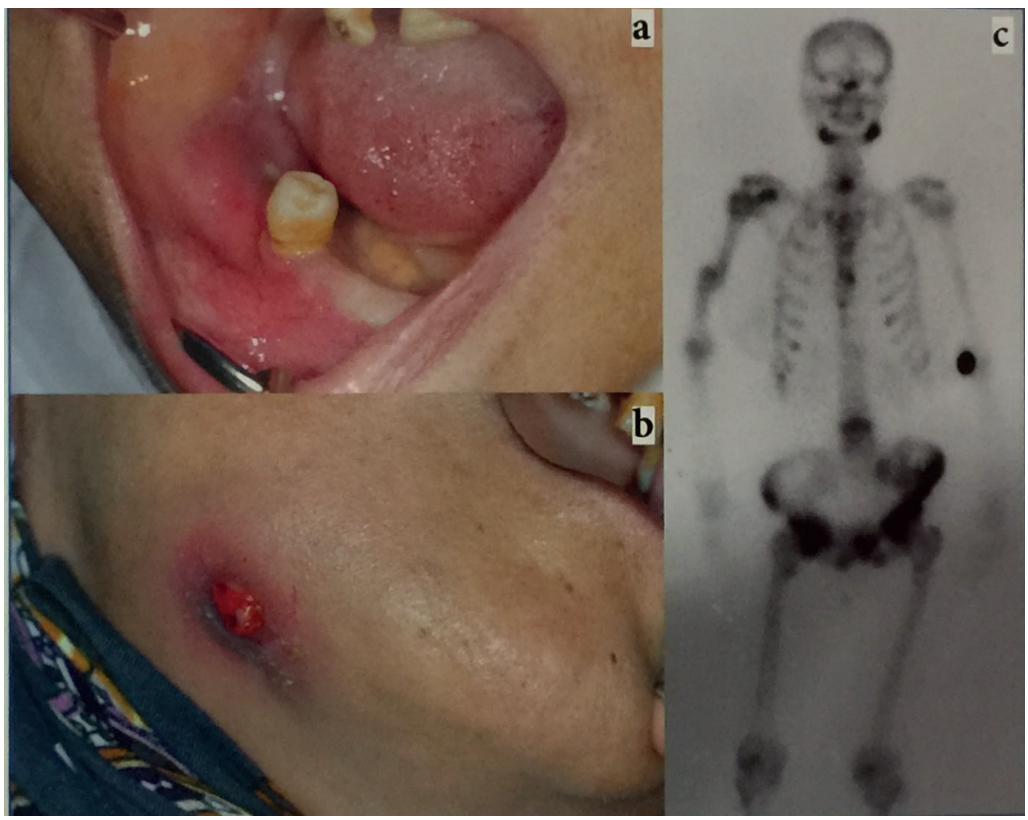


Figure 5. (a)The necrotic bone related i.v. zoledronic acid administration in patient with breast cancer. (b) Extraoral sinus tract of infection (c) Multiple involvement on *scintigraphic examination with* Tc—99.



In patients with bisphosphonates related osteonecrosis, first of all, medical treatment should be started in order to relieve the pain, control the infection in soft and hard tissues and stop the progression of the disease. Antibiotics (penicillin, second generation cephalosporins, macrolides), chlorhexidine mouthwashes as antiseptically should be used to prevent the development of secondary infection and the wound should be washed regularly with serum. The general approach should be to wait for the spontaneous sequestration of the necrotic bone with antibiotic protection without the mechanical intervention (Doğan et al., 2009).

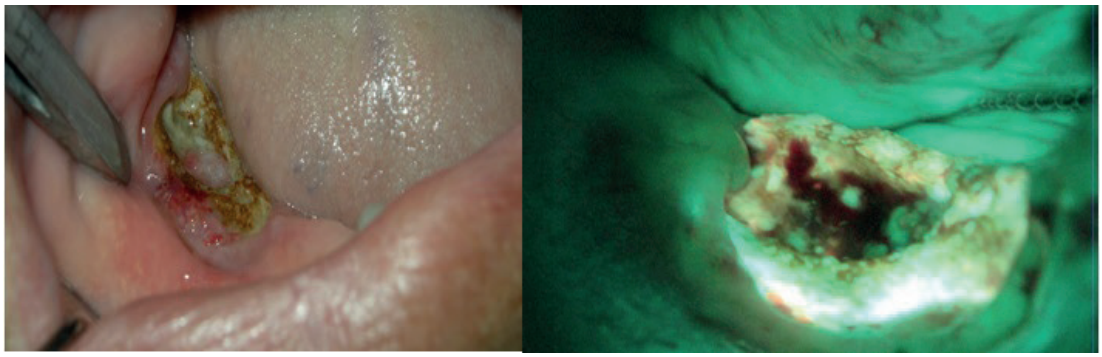
Antifungal treatment should be performed when necessary. Dental treatments such as tooth extraction, surgical procedures and soft tissue interventions including curettage should be avoided. If surgical procedures are required, the vasoconstrictor-free anesthetic should be used and the serum carboxy-terminal telopeptide (CTX), a bone turn-over marker, should be checked to determine the risk of osteonecrosis. If the serum CTX value is 150 pg/ml or more, there is a minimal risk of osteonecrosis, levels of 100-150 pg/ml indicate a moderate risk, while levels of less than 100 pg/ml a high risk. It is necessary to contact the oncologist to discontinue the bisphosphonate (drug holiday) at least three months before the surgical procedure. At the first examination and three months later it is necessary to measure the CTX. Values lower than 150 pg/ml would advise against surgical procedures and an interruption prolonged for another three months (Doğan et al., 2009). However, recent studies have suggested that this marker is not very safe.

4. Approach to Bisphosphonate Related Osteonecrosis

The treatment of osteonecrosis related bisphosphonate-derived drugs depends on the amount of the necrosis bone, whether the infection reaches the cortical part of the bone or the medulla layer. In oncologic patients, whether the systemic condition is suitable for the operation and the disease staging is also important (Özbayrak and Pekiner, 2016).

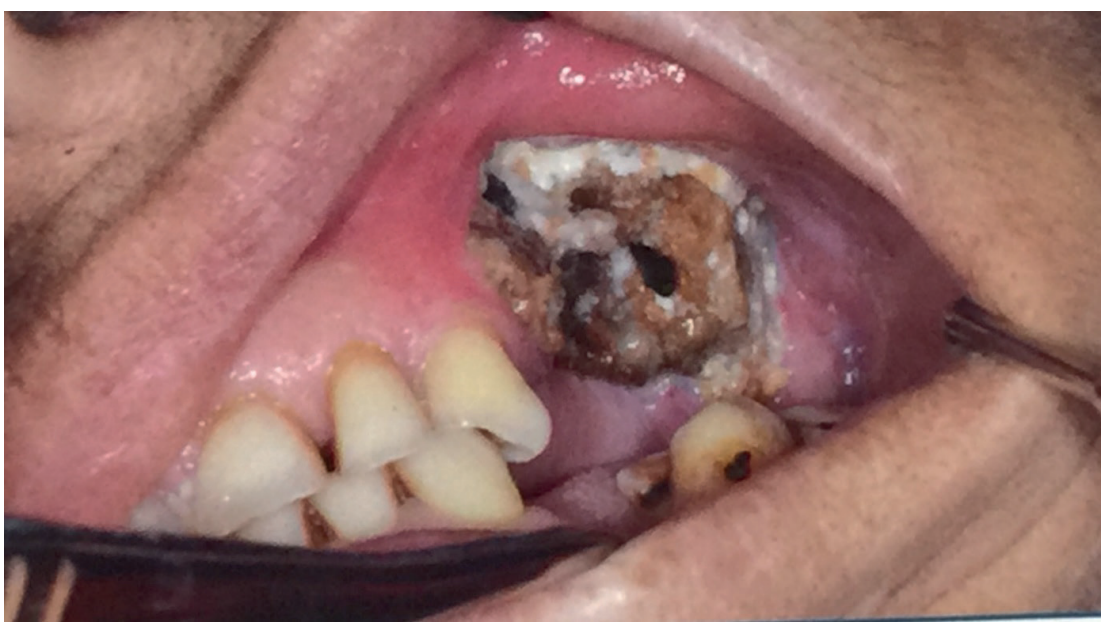
Generally, the small exposed areas can be removed with bone file after oral mouthwash and antibiotic use, and then the soft tissue can be expected to be healed. In total osteonecrosis of the alveolar process, only the resection of the alveolar process, called marginal mandibulectomy/maxillectomy or alveolectomy, may be sufficient. In marginal resections, the necrosis bone is removed until reaches the underlying bleeding living medullar bone and antibiotic treatment is applied. In determining this border, it is advantageous to distinguish the accumulation of pre-operative systemic tetracycline in the intact part of the vessel using the optic device that uses blue light with a 400-460 nm wavelength in order to prevent unnecessary tissue loss (Figure 6). Partial mandibulectomy is performed for infections affecting the entire segment of the mandible. Following the resection of the jaw, a reconstruction plate is placed at the defect to maintain mandible function (Özbayrak and Pekiner, 2016).

Figure 6. The osteonecrosis in patient using zoledronic acid with the diagnosis of prostate cancer and pelvic metastasis. The view of sequester via the optic device that uses blue light with 400-460 nm wavelength (Velscope) and intraoral view (The blue-green color of the light gradually decreases in cancerous tissues).



Especially in advanced oncologic patients, the operation may be contraindicated and it would be appropriate to approach with local precautions. In general, after the infection is suppressed by using double antibiotic pressure and chlorhexidine mouthwash, the mucosa can be healed with a conservative approach by removing the part of the necrotic bone seen in the mouth. Osteo-antral fistula may develop especially in large osteonecrosis involving maxillary sinus floor. In this case, the maxillary sinus should be isolated from the oral environment using a palatal plate (Özbayrak and Pekiner, 2016) (Figure 7).

Figure 7. The oro-antral perforation on the necrotic area extending to the zygomatic crista in a patient using zoledronic acid for breast cancer.



For patients receiving antiresorptive therapy for longer than 4 years, the position paper 2014 of the American Association of Oral and Maxillofacial Surgeons (AAOMS) recommended discontinuation of antiresorptive treatment approximately 2 months prior to invasive dental treatment with preoperative consultation (Ruggiero et al., 2014).

The position paper 2017 of the Japanese Allied Committee on osteonecrosis of the jaw proposes that without discontinuation of bisphosphonates dentists can begin conservative dental treatment. According to their proposal, invasive dental procedures such as tooth extraction can be conducted without discontinuation of antiresorptive treatment following extensive infection control, administration of antibacterial agents in advance and restriction of surgical area (Yoneda et al., 2017). However, no consensus has yet been reached regarding whether bisphosphonates drug holiday before invasive dental treatment is necessary for prevention of MRONJ (Yoneda et al., 2017).

Conclusion

- Good cooperation among physicians, dentists, oncologists, oral and maxillofacial surgens and other healthcare professionals is required to reduce the risk of MRONJ.
- It is important for dentists to be aware of the patients at the risk of this condition.
- The patients should always be carefully examined by a dentist prior to the start of biphosphonates or denosumab or other anticancer agents to remove any focal infection or risk factors.
- For the patients at the risk of this condition, infection control before invasive dental treatments reduces or prevents the occurrence of MRONJ.

Conflict of interests

The authors declare no conflict of interest.

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Case Study

Treatment of Iatrogenic Factor Related Gingival Recession: A Case Report

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Abstract: Recent reports in the literature show that the application of coronally advanced flap in combination with subepithelial connective tissue graft in the treatment of Miller Class II and even III gingival recessions leads to successful results. This case report describes the use of subepithelial connective tissue graft and enamel matrix derivative combination with coronally advanced flap for the treatment of localized gingival recession caused by an ill-fitting crown restoration. The restorative treatment comprised of the retreatment of the endodontic and restorative needs. At 12 months, successful root coverage with 3.5 mm attachment gain was achieved. The patient had minimal discomfort and was satisfied with esthetic results. This case encourages the usage of subepithelial connective tissue graft, enamel matrix derivative and coronally advanced flap combination in localized Miller Class III gingival recessions. Even though the treatment of Miller Class III gingival recessions are challenging due to loss of interproximal bone and soft tissues, the application of subepithelial connective tissue graft in combination with the use ofenamel matrix derivative has shown to be a predictable treatment procedure in esthetic areas forroot coverage and gain of clinical attachment in single Miller Class III recession defects.

Keywords: *Coronally advanced flap; enamel matrix derivative; Miller Class III gingival recession; root coverage esthetic score; subepithelial connective tissue graft*

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1.Introduction

As clinicians one of our aims is to perform biologically integrated and esthetically pleasing restorations. If tooth preparation does not performed properly, trauma to the periodontal tissues (Gracis et al., 2001)

and biological width violation (De Waal and Castellucci, 1994) can occur and lead to the emergence of gingival recessions. Especially in such cases, a good esthetic outcome can be achieved by accurate diagnosis and development of a comprehensive treatment plan. The clinician should always implement both the current knowledge of restorative and periodontal procedures together. Although many techniques have been proposed to treat localized gingival recessions, combination of coronally advanced flap (CAF) and subepithelial connective tissue graft (SCTG) provides more gingival stability in the long term (Cairo, 2017). When there is interproximal bone loss as seen in Miller Class III gingival recessions and naked root surface is wide, additional biological mediator increases the predictability of root coverage. From this perspective, an enamel matrix derivative (EMD) with its angiogenic activity and regenerative capacity is a valuable option for clinicians. It may be expected that the combination of EMD with SCTG will affect periodontal wound healing and regeneration positively (Miron et al., 2016). This case report presents the restorative and periodontal surgical treatment of a patient with gingival recession and high esthetic demands using the CAF combined with SCTG and EMD.

2. Case Description and Results

A 35-year-old female patient applied to our clinic with complaints of poor esthetic appearance and gingival inflammation at the anterior maxilla. The medical history of the patient did not reveal a systemic contraindication for dental treatment. She did not smoke. The patient had undergone a prosthetic treatment that was without harmony and symmetry. After the assessment of clinical and radiographic conditions, patient was diagnosed as localized chronic periodontitis together with localized gingival recession in tooth #11 (Figure 1a). Iatrogenic restorative treatment and microbial dental plaque were defined as etiologic factors.

All clinical parameters were measured at the mid-buccal aspect of the related tooth; probing depth (PD) of 2 mm, recession height (RH) of 3 mm, clinical attachment level (CAL) of 5 mm, recession width (RW) of 3 mm, keratinized tissue height (KTH) of 3 mm, and gingival thickness (GT) of 1 mm. Radiographic evaluation revealed interproximal bone loss and recession defect was classified as Miller Class III (Figure 1b). Root canal of tooth #11 was retreated (Figure 1c), and a temporary crown was placed to allow better healing (Figure 2a). Since a healthy periodontium should be established before any prosthetic procedure, a comprehensive periodontal therapy was performed. The first step of this treatment was oral hygiene instruction and scaling and root planning (initial periodontal therapy). One month after initial therapy re-evaluation was done and low full-mouth plaque (13%) and bleeding scores (12%) were achieved and decided to move on surgical periodontal therapy. Surgical treatment plan was decided as CAF in combination with SCTG+EMD in order to treat the affected hard and soft tissue defects around tooth #11 at the same time, and to improve the level of clinical attachment. After all risks and benefits were explained to the patient, oral and written consents were obtained.

After the application of local anaesthesia, an intracrevicular incision was made at the buccal aspect of the tooth #11 (Figure 2b) and extended to the mesial line angle of tooth #21 and one tooth distally till the mesial line angle of tooth #13. Then, trapezoidal flap was prepared from the line angles and extended to the mucogingival junction reaching the alveolar mucosa in order to position the flap coronally without

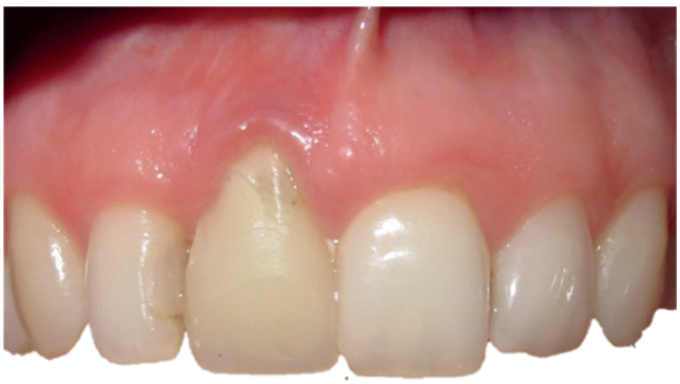
any tension. The flap was elevated in a split-full-split thickness approach (Figure 2c) (Pini Prato et al., 1992). The epithelium on the interdental papilla was de-epithelized for better vascularization. The exposed root surface was conditioned with EDTA 24%, pH 6.7 (PrefGel 0.6 ml gel, Straumann, Basel, Switzerland) for 2 min. Then, the root surface was rinsed with saline. The SCTG, obtained by trap door technique (Figures 2d, 2e) was adapted to cover the root surface to the level of cementoenamel junction (CEJ), and sutured by using sling suture technique with 5-0 resorbable sutures (Pegelak, Dogsan, Turkey) (Figure 2f). EMD was applied (Figures 2g, 2h). Finally, the flap was coronally advanced at least 1 mm coronal to CEJ and sutured to completely cover the SCTG using double sling suture technique with 5-0 non-resorbable polypropylene monofilament sutures (Propilen, Dogsan, Turkey). The releasing incisions were closed with interrupted sutures (Figure 2i).

For infection and pain control, systemic antibiotic (amoxicillin clavulanate, 2×625 mg, 5 days), oral analgesic (naproxen sodium, 550 mg, first day after surgery 2×1, afterwards as necessary), and oral rinse (chlorhexidine solution 0.12% rinse, 3×1 for 1 minute) were prescribed. After 2 weeks, sutures were removed. Four weeks after surgery, temporary crown was revised to achieve better soft tissue contour. At 6 months, permanent restoration was applied.

Post-operative healing was uneventful and oral hygiene remains stable. At 12 months, the recession was almost completely covered with thick keratinized tissue (Figure 3a). However there was still a slight gingival recession (0.5 mm) at tooth #11. An improvement in the thickness and height of the buccal keratinized tissue was observed when compared to baseline. CAL gain was 3.5 mm and this corresponds to a 1 mm reduction in PD and 2.5 mm recession reduction at 12 months. The buccal KTH was 6 mm with 3 mm of KTH increase. GT was increased to 2 mm. Patient satisfaction and esthetic evaluation was also performed by using patient satisfaction score (Mhajan et al., 2007) and root coverage esthetic score (Cairo et al., 2009). Patient satisfaction was assessed by using a three-point rating scale and patient was questioned with regard to root coverage attained, relief from dentinal hypersensitivity, colour, shape and contour of gums, surgical procedure, post-surgical phase and cost effectiveness. This score was detected as 19 out of 21. This result demonstrated that patient was highly satisfied. For the esthetic outcomes of the procedure; gingival margin level, marginal tissue contour, soft tissue texture, mucogingival junction alignment and gingival colour were evaluated. Zero, 3 and 6 points were used for the evaluation of the position of the gingival margin, whereas a score 0 or 1 point was used for each of the other variables. Patient's root coverage esthetic score was found 7 which mean that the esthetic outcome was satisfying.

Figure 1. (a) Clinical view of the patient before initial therapy (b) Baseline radiographical view (c) Endodontic retreatment of #11

a)



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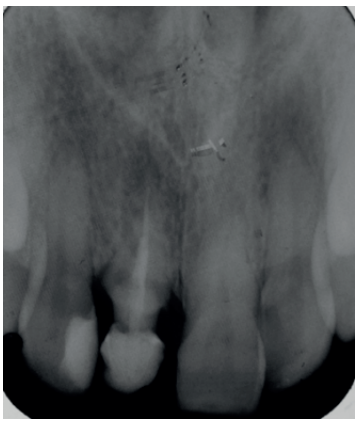

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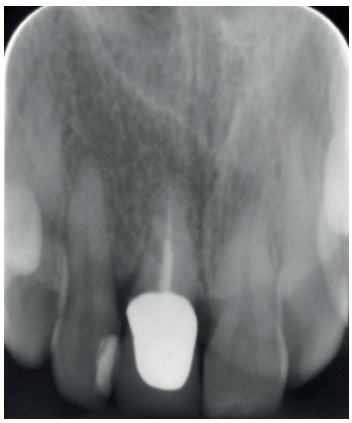
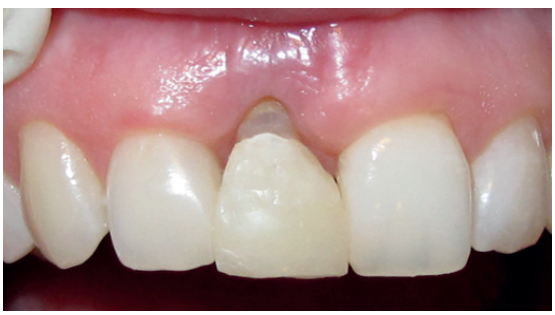
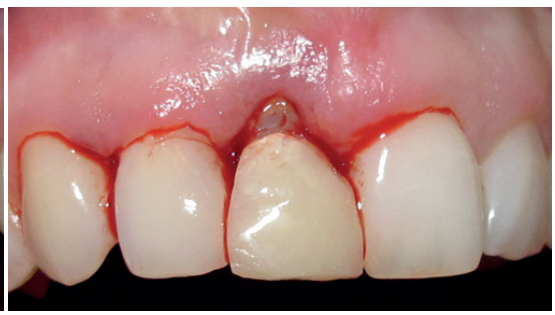


Figure 2. (a) Pre-operative clinical view of recession defect (b) Insicion (c) Flap elevation (d) SCTG harvesting (e) Suturing of the harvesting area (f) SCTG placement (g) EMD application (h) SCTG + EMD (i) Suturing

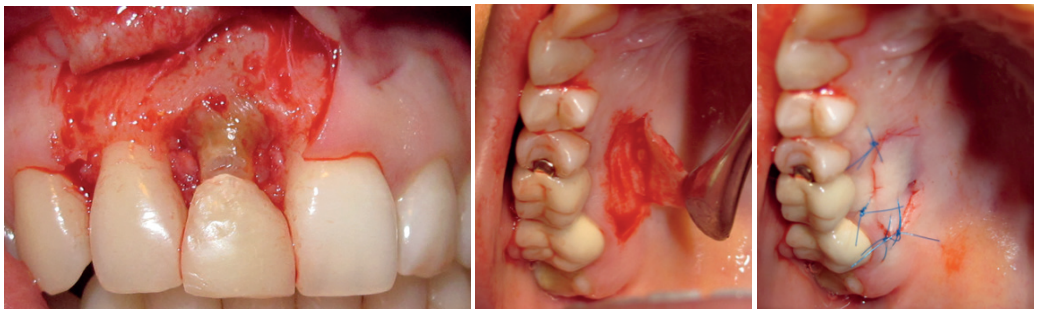
a)



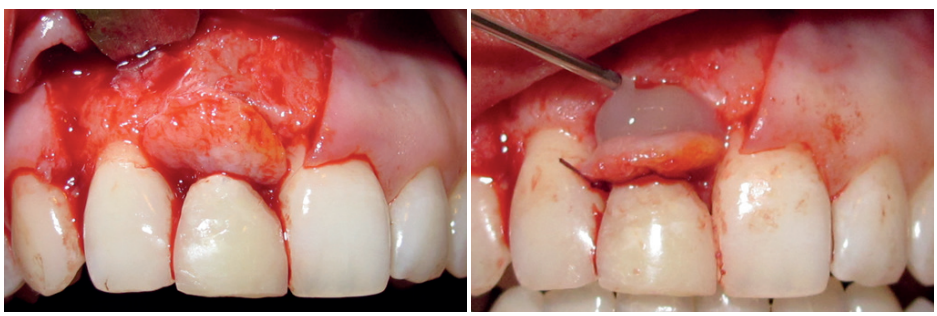
b)



c) d) e)



f) g)



h) j)

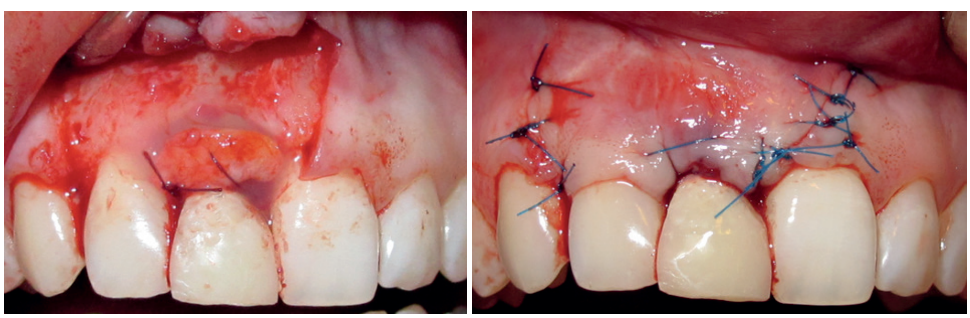
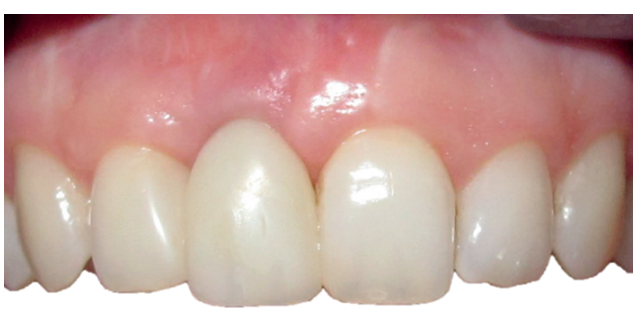


Figure 3. 12 months postoperative clinical view



3.Discussion

The surgical technique described in this clinical case aims to combine two different surgical approaches. Significant recession reduction and CAL gain was achieved. This procedure seems to be a predictable procedure by using SCTG and EMD combination along with CAF for the treatment of single Miller Class III recession at the esthetic zone caused by iatrogenic tooth preparation and improper crown restoration. When the biologic width is intruded by restorations, the periodontium reacts by recreating room between the alveolar bone and the restorative margin to allow space for tissue reattachment. This can result in gingival inflammation, increased probing depths and gingival recession as seen in this case (Bennani et al., 2017). The choice of surgical technique to treat such recessions will depend on different factors; area anatomy, the interdental attachment level and the amount and thickness of keratinized tissue apical and lateral to the recession. The use of EMD+SCTG+CAF has been deeply researched in the treatment of gingival recessions (Henriques et al., 2010, Rasperini et al., 2011, Shirakata et al., 2018). Some studies presented more favourable results with EMD+SCTG+CAF technique whereas other studies did not find any additive effects of EMD to evaluated clinical parameters. Since in this case there was interdental bone loss and wide naked root surface, EMD was also preferred in addition to CAF+SCTG application.

With regard to Miller Class III recessions, data from the literature stated that the accomplishment of CRC was possible with SCTG and CAF application in defects presenting mild interdental attachment loss of 1 to 3 mm (Esteibar et al., 2011, Sculean et al., 2016). It has been suggested that additional grafting can stabilized marginal tissue and provide a scaffold to support wound healing with increasing the thickness of the wound area (Baldi et al., 1999). The clinical results from this case show that EMD used in conjunction with SCTG was successful in the treatment of Miller Class III recession. An incomplete, but at the same time substantial root coverage was achieved (83%). At this point we should keep in mind that, the tooth #11 is a restored tooth and in the literature, the demonstrated results of CRC with Miller Class III recessions are mostly at native tooth.

Considering the results of the present case report, application involving SCTG in combination with the use of EMD seems to be a predictable treatment procedure for root coverage and gain of clinical attachment in single Miller Class III recession defects. However, it is clear that further studies with a larger number of patients are warranted to reach a definitive clinical result.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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Instruction for Authors

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Guide for Authors

The manuscripts submitted to Aurum Journal of Health Sciences are subjected to an editorial review which includes double blind peer reviewing of the manuscript by the experts on the field. Authors should provide a declaration stating that their manuscript has not been published or being considered for publication in any other journal (Please find the Authors Declaration Form in the webpage of the journal. Authors declaration form has to be filled, signed and a scanned version of the filled form should be sent with the manuscript submission). All the submitted manuscripts should adhere the most recent version of the European Community guidelines and Declaration of Helsinki, for humans. The manuscripts that describe experiments which involve research on humans and animals must have an approval of an institutional or local ethics committee. The submitted manuscripts to the Aurum Journal of Health Sciences are screened for authenticity by the Publisher's Office using an authenticity check program for determination of plagiarism and non-ethical situations. Authors could submit their manuscript electronically to the a.jhealthsci@altinbas.edu.tr. Authors who are submitting their work to the Aurum Journal of Health Sciences has to certify that all of the authors of the manuscript accept and confirm the submitted work to the journal.

Types of articles

The Aurum Journal of Health Sciences publishes *research articles, short reports, reviews, case studies* on all aspects of health sciences both in electronic and printed versions. Authors are encouraged to provide proofs of their research results and/or ethical committee approvals as a supporting material which will be published electronically separately with the article.

1. *Research articles:* The manuscripts that are describing findings of an original research in regard to all aspects of health sciences will be published as a "*Research article*". The research articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5.

Introduction; 6. Materials and Methods; 7. Results 8. Discussion; 9. Conclusion; 10. Acknowledgement; 11. Conflict of Interests; 12. References. Manuscripts submitted as a "*Research article*" do not have a wording limit. However, manuscripts that are submitted as "*Research article*" should be more than 5000 words, excluding the tables, figures and references.

2. *Short Reports:* The manuscripts that are describing preliminary findings obtained from an original research or/and results of pre-study performed on a topic in regard to all aspects of health sciences will be published as a "*Short report*". The short report articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Materials and Methods; 7. Results; 8. Discussion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Short report*" should not exceed 5000 words excluding the tables, figures and references.

3. *Reviews:* The manuscripts that are describing critical evaluation of the current situation in the literature and providing future prospects according to current knowledge on a topic in regard to all aspects of health sciences will be published as a "*Review*". The reviews should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Contents; 6. Introduction; 7. Sub-Topics Provided in Contents; 8. Conclusion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Review*" should not exceed 10,000 words excluding the tables, figures and references.

4. *Case studies:* The manuscripts that are describing a critical evaluation of an observed clinical cases in dentistry, medicine and clinical pharmacy will be published as a *"case study"*. The case studies should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Description of the Case; 7. Discussion; 8. Acknowledgement; 9. Conflict of Interests; 10. References. Manuscripts submitted as a "*Case report*" should not exceed 5000 words excluding the tables, figures and references. A written consent of the patient may be required if the case report contains images taken from the patients. All the case reports must contain ethical committee approvals.

Preparation of manuscript and general rules

The manuscripts should be written double spaced in Arial font type and 12 pts font size. Each page should be numbered, and consecutive line numbers should be provided. Title page, authors list and affiliations should be prepared as a separate file. Tables and Figures should also be prepared as a separate file.

Title Page: The title page should contain the full title of the work which should not exceed 200 characters. Abbreviations should be avoided in the title. Main title of the manuscript should be followed by the "*short title"* which should not be longer than 70 characters. Short title should be followed by the list of author names. Author names should be given as name and surname followed by superscript Arabic numbers indicating the affiliations. One author should be designated as the corresponding author and should be indicated in the authors list with the superscript asterix symbol after the affiliation indicator. Author list should be followed by the list of affiliations which indicate the department, institution, postal code, city, country and e-mail(s) of the author(s). Finally, corresponding author full mailing address, telephone, fax and e-mail should be provided. Acknowledgement and Conflict of Interests parts should be given in the title page.

Main text: Main text should be divided into sections and sub-sections using Arabic numerals, starting from the introduction part. Sections should be indicated with bold and non-italic characters. Sub-sections should be indicated with bold and italic characters (as given in example).

Section Example: 1. Introduction

Subsection Example: 2.1. *GC-MS Analysis*

First page of the main text should contain the title followed by a 300-word abstract. Abstract should not contain citations. Abbreviations could be used in the abstract however; full explanation of the abbreviations should be given at the first time that they have appeared in the abstract. Abstract should briefly summarize the study. Abstract should contain the following information: 1. Purpose/Aim of the study; 2. Materials and methodology used in the study; 3. Key results obtained in the study; 4. Conclusion remarks. Abstract part should be followed by 6 keywords that describe the work. Keywords should be separated from one another with a semicolon.

Depending on the type of article following parts should be given in the main text. Introduction part in the manuscript should contain a brief explanation of previous studies, aim of the current study and reasoning of the study. Materials and Method part should be given in full detail allowing replication of the performed experiments/clinical studies/technical studies by other scientists. In materials and methods section all the instruments, chemicals used in the study should be explained by their brand and model. Results, should be described without any comments. Discussion and conclusion parts should not contain any speculations. A clear and concise discussion and conclusion remarks should be given.

Acknowledgement

Authors should indicate any acknowledgement related to the study in this part.

Conflict of Interests

Authors should clearly indicate any kind of conflict of interests for the study in this part. If the authors do not have any conflict of interests, they should indicate "Authors declare no conflict of interests".

Tables & Figures

The authors should indicate the position of Tables and Figures in the text by indicating the Title of the table (as given in the example). All figures should be provided as a tiff file with at least 300 dpi resolution. The images given as figures should be authentic, no manipulations should be done. Color figures are welcome in the journal and does not require a publication fee.

Example: Figure 1. ^{1}H-NMR spectrum of *Ulubelenolide*.

The figures and tables should be given as a separate file. Each table and figure given should contain a title and if required footnotes should be given. Each figure and table given should be self-explanatory.

Reference Format

Citation of references in the text

Authors must check their manuscript that every reference cited in their text should also be in the given reference list and every reference listed should be in cited in the text. Citations of unpublished results and personal communications should be avoided. Citations of literatures that were accepted by a journal and which have doi number, issue and page numbers could be cited in the text however, authors should indicate that this work is "*in press*". The citations of the web pages should be avoided. The citations in the text should adhere to the following style.

Cited reference which have a single author: (author's last name, year of publication)

Example: (Biyikoglu, 2017)

Cited reference which have two authors: (last name of the first author and second author, year of publication)

Example: (Biyikoglu and Polatoglu, 2017)

Cited reference which have three authors or more: (last name of the first author et al., year of publication)

Example: (Polatoglu et al., 2017)

Cited references which have the same first author(s) that were published in the same year: (last name of the author, year of publication and uncapitalized letters for separation)

Example: (Biyikoglu, 2017a; Biyikoglu 2017b)

Cited references as lists: The references that are going to be given as a list in a single parentheses should be first arranged alphabetically than chronologically.

Example: (Biyikoglu 2017a; Biyikoglu 2017b; Polatoglu et al., 2013)

Cited references given in text: If author names are going to be mentioned in the text for the citation than it should be given as: "….Polatoglu et al. (2013)……"

Examples: "……..Polatoglu et al. (2013) have indicated….

"……Biyikoglu (2017) demonstrated that……"

Reference formatting

The reference formatting should be given according to the following style (APA). DOI numbers should be given after the reference if available.

Reference style

Reference to a journal publication:

Polatoglu, K., Demirci, F., Demirci, B., Gören, N., Baser, K. H. C. (2010). Antibacterial activity and the variation of Tanacetum parthenium (L.) Schultz Bip. essential oils from Turkey. Journal of oleo science, 59(4), 177-184. https://doi.org/10.5650/jos.59.177

Reference to a book:

Preedy, V. R. (Ed.). (2015). Essential oils in food preservation, flavor and safety. 1st Ed., Academic Press, Elsevier, Oxford, UK.

Reference to a chapter in an edited book:

Polatoğlu, K., Karakoç, Ö. C. (2015). Biologically Active Essential Oils against Stored Product Pests. 1st Ed., In Preedy, V.R. (Eds.), Essential Oils in Food Preservation, Flavor and Safety. Academic Press., Elsevier, Oxford, UK, pp. 39-59.

Reference to a website:

National Cancer Institute, A success storyTaxol® (NSC 125973) https://dtp.cancer.gov/timeline/flash/success_stories/s2_taxol.htm (accessed 14 December 2017)

Reference to a Thesis:

Knight, K.A. (2011). Media epidemics: Viral structures in literature and new media (Doctoral dissertation).

Abbreviations

Full explanation of the abbreviations should be given at the first time that they have appeared in the text. Title should not contain any abbreviations. After the explanation of the abbreviations are given in the text authors could use abbreviations throughout the text.

Example: "…..Acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes were ……."

Chemical and Biological Nomenclature

The names of the biological organisms should be given in full of the author name at the first time they appear in the text. The genus and species names should always be written in italics. Authors could use the short name of the organism after the full name was indicated. Local names of the organisms could be mentioned however, throughout the manuscript these organisms should be referred to with their binominal names.

Chemical compounds should be preferably named according to the IUPAC nomenclature.