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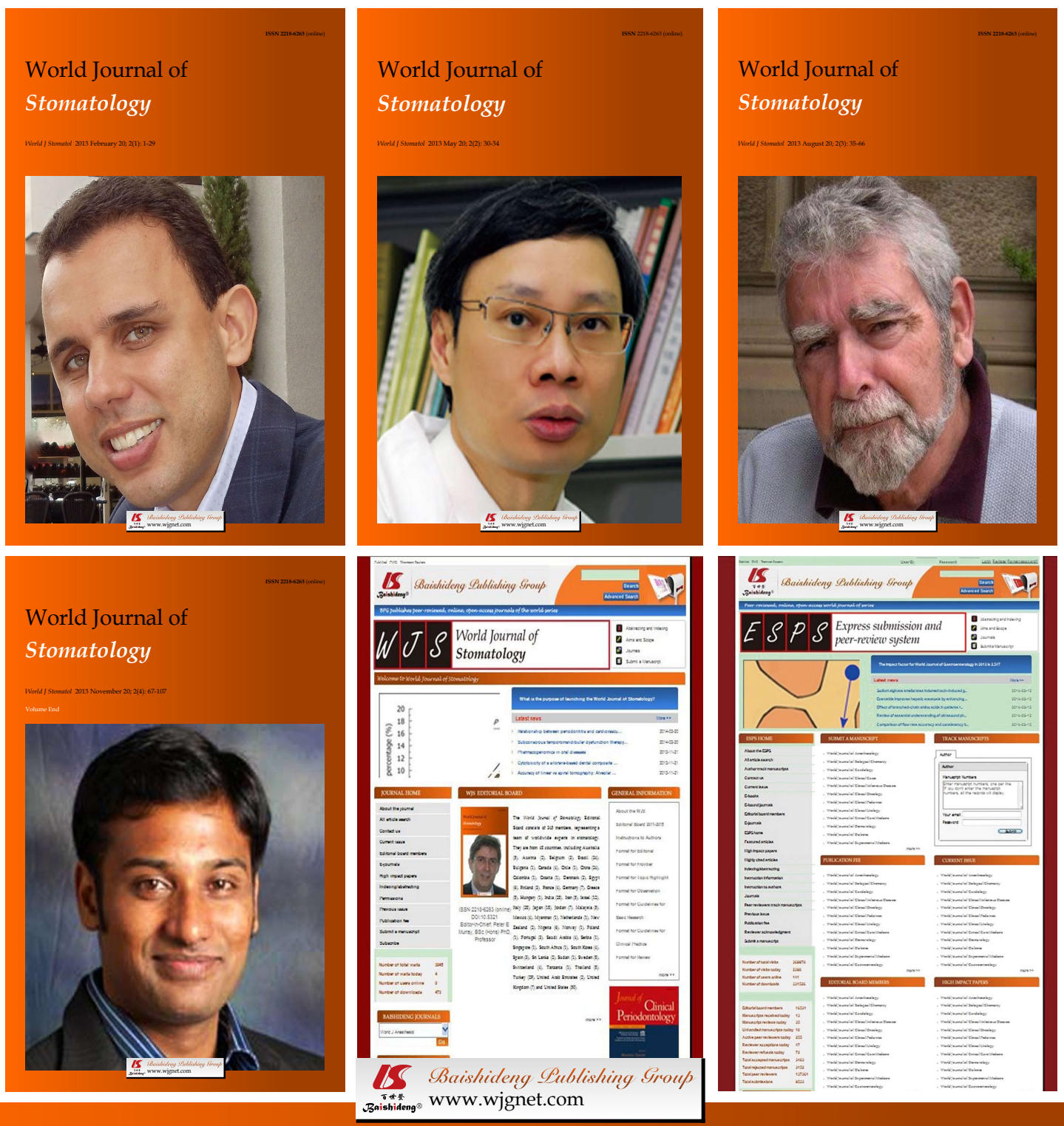

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FRONTIER

Risk aspects of dental restoratives: From amalgam to tooth-colored materials

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Abstract

Dental materials' choice of patients has considerably changed. Whereas cast gold and amalgam have been the predominant biomaterials for decades, today tooth-colored materials like resin-based composites and ceramics are more and more successful. However, are we going to replace a good but biologically questionable material (amalgam) with an equal material (resin composite) being more esthetic but also biologically questionable? For amalgam, long-term clinical studies reported some significant hints that in single cases amalgam may be a health hazard for patients, finally Norway banned amalgam completely. The main advantage of a resin-based composite over amalgam is its tooth-like appearance and more or less absence of extensive preparation rules. For many years it was believed that resin-based composites may cause pulpal injury. However, pulpal injury associated with the use of resin-based composites is not correlated with their cytotoxic properties. Nevertheless, resin-based composites and other dental materials require rigorous safety evaluation and continuous monitoring to prevent adverse events similar like with amalgam. Because of non-biocompatible pulp responses to resin-based composites and amalgam, they should not be placed in direct contact with the dental pulp. The less dentin remaining in the floor of preparations between resin-based composites or other dental materials is more likely to cause pulpitis. Percentage of patients and dental practitioners who display allergic reactions is between 0.7% and 2%. The release of cytotoxic monomers from resin-based materials is highest after polymerization and much lower after 1 wk. Substances released from resin-based composites have been shown to be toxic in cytotoxicity tests. Nevertheless, *in vitro* cytotoxicity assays have shown that amalgam has greater toxic effects than resin-based composites, sometime 100-700-fold higher. Altogether, the risk of side-effects is low, but not zero, especially for dental personnel.

Key words: Exposures; Restoratives; Amalgam; Resin-based composites; Adhesives

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INTRODUCTION

The choice of dental materials has considerably changed

during the last 20 years[1-3]. In former times, cast gold and amalgam have been the materials of choice for decades[4]. However, after amalgam was alleged to be inacceptably toxic and simultaneously esthetic demands of patients were growing, tooth-colored materials like resin-based composites and ceramics took more and more parts of this huge market[5].

In terms of biocompatibility and exposure, cast gold may be still the best restorative material, however, it is non-esthetic when it is used in visible areas such as premolars and also here some health concerns in terms of gold allergies are present[6]. Furthermore, the gold prize considerably increased from < $ 200 to > $ 1000 per ounce during the last decade which is consequently also transferred to restoration costs and therefore being detrimental for cost effectiveness as well. Other highly biocompatible materials like phosphate or glass ionomer cements are too brittle and therefore not able to withstand intraoral occlusal forces in deciduous and permanent teeth over time[1,2].

Today's restorative trend clearly answers the question "black or white?" by more and more moving from metallic amalgam to resin-based composites[5]. The same is true for bonded all-ceramic restorations such as ceramic inlays and onlays, because they have to be adhesively luted with the same adhesives and resin-based composite luting cements. So we face the interesting question whether we are replacing a clinically good but biologically questionable material (amalgam) with an equal material being more esthetic (resin composite) but also (or even more?) biologically questionable.

AMALGAM

Amalgam is one of the by far most successful dental restoratives which has been used all over the world since more than 150 years[7-10]. Long-term data are sufficient and long-term costs due to repair, refurbishment and tooth hard tissue loss during replacement are favourable[1-3,11-15]. Disadvantages are a compromised esthetic appearance due to an argentic to black color and especially biocompatibility concerns[7,16-24]. Dental silver amalgam consists of 50% mercury (in a complex mixture of copper, tin, silver, and zinc) and therefore this material was always suspected to be a considerable hazard for both patient and environment[25-35].

In the literature of the field, two opposing groups are identified: Primarily toxicologists are arguing against the health risks of mercury vapor being released from amalgam restorations and potentially threatening health of both patients and dentists and moreover polluting the environment by dental mixing and application processes[25-50]. On the other hand many authors with clinical dental background repeatedly state that amalgam per se is one of the most successful restorative materials[3,5,8,11,13,14,23,24,51-65]. So what is the real threat with amalgam? It is common knowledge that high-dose exposure to elemental mercury vapor cause several diseases like emotional dysfunction[53]. However, it is not fully understood to the date whether smaller amounts like being released from amalgam restorations are a considerable health hazard as well[51-53,66,67].

In a retrospective cohort study involving 20 000 participants over 20 years (1977-1997) in the New Zealand defense force[28]. The cohort was linked with morbidity records by use of a time-varying exposure unit of 100 amalgam surface-years. Multiple sclerosis had an adjusted hazard ratio of 1.24, but there was no association with chronic fatigue syndrome (0.98), or kidney diseases[28]. Also Aminzadeh *et al*[25] reported some hints for a possible correlation of amalgam restorations and multiple sclerosis, however, also stating that more clinical studies are needed.

One of the most intensive clinical trials so far was the New England's Children Amalgam Trial (NECAT) giving clinical result. 534 children (6-10 years old) with carious primary molars received either amalgam or resin composite restorations. Evaluated parameters were neuropsychological outcome (Full-Scale IQ score, General Memory Index, Visual-Motor Composite of the Wide Range Assessment of Visual Motor Abilities) and renal glomerular function with no statistical differences between resin composite and amalgam groups in any of the investigated criteria[51,52,68]. Furthermore, parent-completed child behavior checklists and children's self-reports were collected. Children's psychosocial status was evaluated in relation to three indices of mercury exposure: treatment assignment, surface-years of amalgam, and urinary mercury excretion. Again, there was no evidence that exposure to mercury from dental amalgam was associated with adverse psychosocial outcomes[53]. In another part of NECAT, longitudinal amalgam exposure data in children randomized to amalgam restorations were analyzed. Amalgam and U-Hg were moderately correlated with the total of amalgam surfaces having been a good predictor of current U-Hg and posterior occlusal surface-years for cumulative U-Hg. One additional amalgam surface caused a 9% increase in current U-Hg, and one posterior occlusal surface-year resulted in a 3% increase in cumulative U-Hg excretion[61]. Finally it could be shown that daily chewing gum use resulted in higher urinary Hg levels[69].

Halbach *et al*[56] measured internal exposure to amalgam-related mercury in plasma and erythrocytes after amalgam removal and estimated the amalgam-related absorbed dose in 82 patients. Post-removal steady-state Hg concentrations were taken for 18 mo for three groups: Removal of the fillings/removal and non-specific detoxification/health promotion program without removal. After amalgam removal, inorganic Hg was decreased, leveling at 27% of pre-removal levels after 60 d. Organic Hg in plasma did not change. Organic Hg in red cells of group A was lower in the early post-removal phase and higher in the late post-removal phase, being higher than the pre-removal control. A protracted increase in organic Hg was also found in red cells of group B after 60 d. In all groups, time profiles of urinary concentra-

tion and excretion of total-Hg were similar to those of inorganic-Hg levels in plasma. It was estimated that the amalgam-related inhalation and ingestion of Hg species were within the limits proposed by the World Health Organization (WHO), Agency for Toxic Substances and Desease Registry, and Environmental Protection Agency. The integrated daily Hg dose absorbed from amalgam was estimated < 3 μg for an average number of fillings and 7.4 μg for high amalgam load, with 30 μg being the tolerable dose according to the WHO[56].

On the other hand, there is no doubt that amalgam restorations release small amounts of mercury during clinical service which is absorbed by several body tissues in human subjects[32-35,64,70-75]. The daily dose is found to be 14% of the threshold above which observable adverse neurological symptoms are expected[75]. It has reported that methyl mercury and inorganic mercury levels in blood and cortex of autopsy bodies with a significant correlation between methyl mercury in blood and occipital cortex. Inorganic mercury in blood and occipital cortex, as well as total-Hg in pituitary and thyroid were strongly associated with the number of dental amalgam surfaces at the time of death[46,67]. Mutter *et al*[33] repeatedly stated that some of the clinical studies reporting low to no risk connected with dental amalgam may be methodically flawed which may lead to inadequate conclusions about the safety of dental amalgam. He also identified mercury vapor as potential reason for autism or Kawasaki's disease[34,35,76]. It is also controversially discussed whether carbamide peroxide tooth bleaching agents lead to an increased release of mercury[77,78].

Another important point in the amalgam issue is occupational exposure for dentists and dental nurses[26,31,79-87]. It was found that a correlation between total Hg-U and duration of dental practice exists[87]. However, a cytogenetic damage in oral health professions dealing with amalgam was not reported[26,87]. Farahat *et al*[82] showed that dental staff have significant exposure to mercury vapor, furthermore indicating a negative impact of mercury on thymus gland functions[82,87]. Jones *et al*[84] investigated possible residual adverse effects from occupational mercury exposure in dentistry in 115 graduates of a dental nurse school from 1968-1971 because 30 years ago, dental nurses worked with amalgam without protective gloves or a ventilation system, resulting in chronic mercury exposure. Significant differences were found in current health experience and reproductive health, especially early hysterectomy experience. Reporting of Occupational Overuse Syndrome was strongly positively correlated with years of work.

Finally, also environmental aspects of mercury pollution by amalgam waste of dental practices and clinics have to be considered. Mercury occurs in nature as sulfides and in some minerals. All over the world every year 20 000-30 000 tons of mercury are discharged into the environment. Less than 50% of freshly triturated amalgam is inserted in cavities, more than 50% is waste. Extracted teeth with preexisting amalgams, amalgam-contaminated capsules and cotton rolls are discharged with the solid waste. However, dental mercury contamination makes only 3%-4% of global mercury being insignificant compared with industrial pollution[30,80,88,89]. With proper amalgam separators it could be even more reduced[30,80,88,89].

Despite all hints towards side-effects caused by mercury vapor of dental amalgam restorations, unproportionally many patients suffer amalgam incompatibility. Gottwald *et al*[55] conducted an interdisciplinary case-control study with special focus on toxicological, allergic, psychological and psychiatric aspects. Patients with amalgam-associated complaints (n = 40) were compared to amalgam bearers without complaints (n = 40) regarding quantity, surface area and quality of amalgam fillings, mercury load in blood and urine, allergy examination, and psychometric assessment with questionnaires noting coping strategies, interpersonal problems and self-consciousness. Patients and controls did not reveal different mercury concentrations in body fluids with patients having higher levels of psychic distress, higher incidence of depression and somatization disorders as well as different styles of coping with anxiety compared to controls. So the theory of amalgam-related complaints as an expression of underlying psychic problems was confirmed. A socio-economically important issue is that a ban of dental amalgam would also have some economic impact. Beazoglou *et al*[90] calculated the economic costs of an amalgam ban in the United States with total expenditures for restorations increasing from $ 46.2 billion to $ 49.7 billion and with consequently 15 444 021 fewer restorations inserted per year. An estimated first-year impact of an amalgam ban means an increase in expenditures of $ 8.2 billion.

Altogether it can be summarized that long-term clinical studies primarily demonstrated that amalgam can be safely used for patients, dental staff, and environment. However, there are some significant hints that in single cases amalgam may be a health hazard for patients. From 2008, Norway banned amalgam completely which is another hint[58]. So, amalgam remains an excellent restorative material with centuries of clinical success and decades of significant problems in biocompatibility.

RESIN-BASED COMPOSITES

A remarkable change in restorative dentistry has been the dramatic drop in the use of amalgam to restore teeth[91]. Patient and practitioner demand for a tooth-colored material as an alternative to amalgam was addressed in 1955 by Dr. Buonocore who described the use of a plastic material to restore teeth[92]. Later in 1950s, the first tooth-colored direct restorative material called Sevitron was produced by L.D. Caulk[93]. In the 1960s, several resin-based composite dental restorative materials (resin-based composites) were introduced[94]. The main advantage of a resin-based composite over amalgam is that it can be made in a wide range of tooth colors allowing the almost invisible restoration of teeth. However, the benefits of

resin-based composites in comparison with amalgam and other dental materials have proved to be controversial. Normally resin-based composites can be used to restore teeth and repair or replace failing restorations with less removal of vital tooth structure in comparison with amalgam[95].

Unlike amalgam, resin-based composites must be bonded to teeth using an adhesive, which makes them more expensive and more technique-sensitive. Without meticulous placement, resin-based composite restorations can fail quickly. Nevertheless, even with the most meticulous placement, the longevity of resin-based composite restorations placed in posterior teeth has been shown to be significantly less than amalgam restorations[96]. The main reasons for the inferior clinical longevity of composite restorations in comparison with amalgam are marginal discoloration and a loss of adhesion[97].

Resin-based composites shrink by approximately 5% upon light-curing, which can create gaps for bacterial microleakage along the cavity margins[98]. These examples indicate that many of the problems patients have suffered with resin-based composites does not appear to be directly caused by the chemicals within the formulation of the material, but because of the shortcomings of the material when it is used to restore teeth. The shortcomings of resin-based composites, particularly their polymerization shrinkage, are an active area of research and new lower shrinkage materials are under development to help improve their clinical performance similar to amalgam restorations.

The earliest resin-based composites had the worst longevity because they were prone to breakage and leakage due to their weak compressive strength[99]. The initial techniques to etch enamel to bond dental restorations were also not very successful, so many restorations suffered a loss of adhesion and were lost[100]. Many clinicians were initially reluctant to bond to dentin because they feared the high acid content of the etchant would cause a necrosis of underlying pulp tissue[101]. Subsequently, it was discovered that the buffering capacity of dentin, along with an improved quality of sealing to reduce microleakage, reduced the pulp irritation beneath resin-based composite restorations[102]. As research progressed, the concept of the "hybrid layer" was created to explain the physical and chemical interactions of the adhesive, resin-based composite, and tooth structure[103]. The "hybrid layer" concept has proved to be useful to develop research strategies to increase the quality of sealing and bonding of resin-based composites to tooth structure[104]. Improvements to the process of accomplishing resin-based composite bonding to tooth structure progressed through a number of "generations". Each new generation of resin-based composite materials have had improved bonding and physical properties which are beneficial to patients through their increased longevity[105]. The current, 7th generation of resin-based composite adhesives can accomplish very high bond strengths to tooth structure[106]. The newest generations of "one-step" resin-based composite materials are generally easier for practitioners to use, and help reduce the exposure of patients to failed restorations.

For many years it was believed that the toxicity of the chemicals in the resin-based composite materials was responsible for pulpal injury. However, pulpal injury associated with the use of resin-based composites could not be correlated with their cytotoxic properties[107]. The discovery of the effect of bacterial contamination on the vitality of the tooth pulp, was a major milestone in dental research. In general, resin-based composites and other dental materials do not provide a hermetic seal with the tooth structure. Bacterial leakage may subsequently occur. The presence of bacteria and their toxic products can evoke an inflammatory response in the underlying pulp. Suh *et al*[108] demonstrated that the growth of bacteria in cavity restorations was directly correlated with pulpal inflammatory responses in the adjacent pulp tissue. As yet no permanent filling material has shown to consistently provide a perfect marginal seal, so leakage and bacterial contamination are always a threat to the integrity of the pulp. Therefore, the antibacterial properties of restorative materials are of considerable importance, and this explains the clinical success of some cytotoxic restorative materials, such as zinc oxide eugenol[109]. Despite these findings, it must be acknowledged that generally it is preferable to use dental materials which have the least potential to be toxic to patients and dental professionals. Similar to amalgam, resin-based composites and other dental materials require rigorous safety evaluation and continuous monitoring[110] to prevent adverse events.

Dentin and enamel have different physical properties and elemental compositions which have complicated the resin-based composite bonding to tooth structure[111]. It was discovered that the inclusion of hydrophobic monomers in adhesives could not penetrate the aqueous environment of demineralized dentin. Thus, methacrylate-based priming agents were used to create a permeable interface for the formation of a hybrid layer[112] which can increase micromechanical retention of the resin-based composite[113]. Thus, the need for "wet bonding" arose, and techniques for preparing the interface for increasingly hydrophobic monomers were developed[114]. Wet bonding systems have been successful[115]. However, they require the handling of multiple components which must be used in multiple steps. To facilitate the ease and speed with which bonding can be accomplished, the latest generation of "one-step adhesive systems" have been introduced which don't have a separate acid etching step. Instead, acrylic resin monomers themselves provide the acidity needed for demineralization and simultaneously penetrate exposed and uplifted collagen fibrils[116]. A dental composite typically consists of a resin-based oligomer matrix, such as a bisphenol A-glycidyl methacrylate (Bis-GMA) or urethane dimethacrylate (UDMA), and an inorganic filler such as silicon dioxide silica. Compositions vary widely, with proprietary mixes of resins forming the matrix, as well as engineered filler glasses and glass

ceramics. The filler gives the composite wear resistance and translucency. A coupling agent such as silane is used to enhance the bond between these two components. An initiator package (such as: Camphorquinone, Phenylpropanedion or Lucirin) begins the polymerization reaction of the resins. A catalyst is added in varying concentrations to control the speed of polymerization[117]. Resin-based composite materials are all capable of causing moderate to severe cytotoxicity when placed in contact with *in vitro* cell lines[118]. Resin-based composite materials may also cause severe pulp necrosis when used for direct-pulp capping[119]. The migration of adhesive and resin-based composite particles into pulp tissue can stimulate inflammatory responses[120]. Because of these non-biocompatible pulp responses to resin-based composites and amalgam, they should not be placed in direct contact with the dental pulp. A biocompatible liner such as $Ca(OH)_2$ or preferably; mineral trioxide aggregate (MTA) must be used as a liner to help prevent unfavorable responses to direct pulp capping with resin-based composite[121] or amalgam. An MTA or $Ca(OH)_2$ liner is not needed in shallow indirect pulp capping restorations because the buffering effect of dentin can prevent the diffusion of chemicals from resin-based composites and amalgam from entering the pulp tissue, particularly when the dentin thickness is above 0.5 mm[122]. The less dentin remaining in the floor of preparations between resin-based composites or other dental materials is more likely to cause pulpitis[122].

A number of local and systemic reactions to resin-based composite materials have been reported. The incidence of patients and dental practitioners who display allergic reactions is between 0.7% and 2%[123-126]. The main source of cellular and molecular cytotoxic injury from resin-based materials is claimed to be the leaching of unpolymerized monomers from the restoration during and after polymerization[127] which can reduce pulp vitality and cause a retraction of the gingival margin[128,129]. The release of cytotoxic monomers from resin-based materials is highest after polymerization and much lower after 1 wk[130]. Which may suggest the health risks to patients and practitioners are highest when in contact with newly polymerized resin-based composite materials, and the health risk diminishes over time.

Erosion and saliva degradation of resin-based composites may cause the release of leachable substances. Human-saliva derived esterases can biodegrade resin-based composites, causing the release of (Bis-GMA) monomers and (UDMA-type) comonomer[131]. The substances released from resin-based composites, particularly the (Bis-GMA) monomers have been shown to be toxic in cytotoxicity tests[132]. The presence of leached compounds is dependent on the formulation of resin-based composite[133]. The more flowable resin-based composites are more toxic than the traditional resin-based composites[134]. The relative *in vitro* cytotoxicity of resin-based composite monomers measured using a bromodeoxyuridine assay discovered that the Hg^{2+} amalgam component was four-fold more toxic than Bis-GMA to human gingival fibroblasts[135]. Almost all the *in vitro* cytotoxicity assays have shown that amalgam has greater toxic effects than resin-based composites, sometime 100-700-fold higher[136]. A problem is the general lack of resin-based composite biocompatibility data in comparison with amalgam. The results from systemic toxicity tests of resin-based composites do not indicate any unacceptable risk to the patient's general health[137]. The *in vitro* screening of some components of resin-based composites are mutagenic[138]. Due to the limitations of the *in vitro* genotoxicity test systems and the comparatively high concentrations needed to elicit the reactions, no unacceptable risk can yet be derived from those data for the patient[139]. Most of the available data suggests that amalgam is relatively more hazardous to patients and dental professionals, than resin-based composites.

Skin and mucosa which come into contact with resin-based composites and bonding agents can become slightly inflamed which is commonly observed as a reddening of the affected area. However, if a patient or dental professional is allergic to a compound within the resin-based composite their reactions may be more severe and allergic irritant contact dermatitis can be observed. Contact urticaria, pigmentary changes, and photoallergic contact dermatitis may occasionally occur. Rarely other health effects, such as respiratory and neurologic signs and symptoms have been reported, but none have been linked to dental resin-based composites[140]. The concentrations are probably too minute to cause systemic reactions[137]. The most common resin-based composites to cause contact dermatitis, are (meth)acrylics, polyurethanes, phenol-formaldehydes, polyesters, amino resins (melamine-formaldehydes, urea-formaldehydes), polyvinyls, polystyrenes, polyolefins, polyamides and polycarbonates[140]. Contact dermatitis usually presents on the hands, fingers, and forearms, while facial, eyelid, and neck involvement may occur through indirect contact, *e.g.*, *via* the hands, or from airborne exposure[140]. Patch testing with commercially available materials is important for a diagnosis of an allergy[141]. In some countries, occupational dermatoses are relatively common among dental staff, sometimes entailing occupational disability and re-schooling[142]. The risk of occupational dermatoses can be reduced by the development of new bonding techniques and careful risk-benefit assessments in the formulation of new dental composites. To protect patients from potential hazards of light-cured monomers released from resin-based composites it is important to use an effective curing unit and to applying the light-curing for the recommended length of time[142]. To protect dental professionals from the potential hazards of monomers released from resin-based composites, gloves should always been worn to prevent direct skin contact.

THE RISK ASPECT IN RESTORATIVE DENTISTRY

Dental restorative materials represent the most frequent

replacement materials in the human body[143]. Despite that fact, biocompatibility issues regarding dental materials (especially amalgam) have not been scientifically evaluated until the early 1980s[141]. During the last two decades, however, amalgam lost its unique feature because adhesively bonded resin composites got suitable even for stress-bearing posterior restorations[144]. The paradigm shift towards minimally invasive restorations additionally supported this trend[145]. However, in many cases there is almost no patient or dental staff knowledge of hazards by the use of dental restoratives[146]. Furthermore it is of significant interest whether recently used dental materials changed the use-risk ratio.

Fundamental judgement tool of dental materials is a risk analysis. Schmalz *et al*[145] defined the term "risk" concerning biocompatibility of dental restoratives as "the probability of a side effect and the severity of that side effect". Risk analysis implies the description of indication ranges of a medical product, analysis of tissue exposure, and potential hazard[147]. So risk analyses try to determine the probability and severity of side effects for human health by exact knowledge of their composition. The consecutive risk assessment clarifies under estimation of usefulness and risk, whether a medical product may enter the market. Here it is decisive to compare the advantages of the material with the frequency and severity of side-effects[148]. In restorative dentistry, primarily a potential hazard by release of ingredients is discussed. Dental biomaterials are medical products with medium hazard potential. This means that clinical investigations are not mandatory in Europe, manufacturers just have to meet minimum requirements[147]. Especially in the post-amalgam era in the middle of the 1990s, some restoratives diminished from the market because minimum requirements were not achieved (Figure 1)[149].

Systematic epidemiological studies concerning frequency of side-effects with dental biomaterials are missing. Mjör[147] reported possible side-effects with different materials with 13 325 sessions (done by 137 dentists) and 24 cases of subjective discomfort, 7 cases of acute nature, and 15 cases of long-standing effects. In eight cases, amalgam was the reason for patients' complaints[150]. So altogether the risk of side-effects is low, but not zero[148].

Dental personnel is much more under risk than patients. Geukens *et al*[148] observed 13 000 patients with contact dermatitis. In 31 patients (meth) acrylates were responsible for the complaints, and almost 50% of these group was working as dentist or dental nurse or dental technician[151]. Unfortunately, latex or vinyl gloves do not guarantee for safety due to their permeability at least after some minutes. This should be one of the reasons that dental personnel reveal increased rates of contact dermatitis of the fingers or allergic reactions following contact with monomers[152-154]. Thus, it is clearly recommended to completely avoid contact with unpolymerized resins[147].

Side-effects of dental materials are primarily of a local nature (*e.g.*, gingivitis, mucosal alterations, pulpitis, *etc.*) or allergic (type I : immediate reaction or type IV: delayed reaction). Contact allergies have been observed for nickel

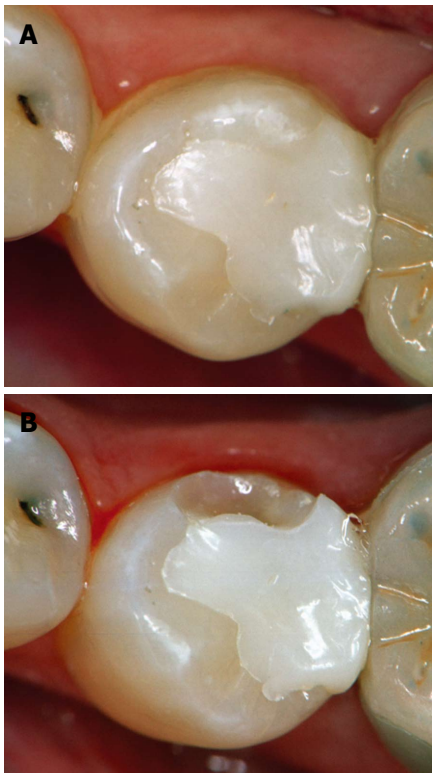

Figure 1 Some restoratives diminished from the market. A: Ariston restoration in lower second premolar at baseline; B: Due to a 2% linear expansion, 18 mo of clinical service were enough to disrupt the lingual cusp.

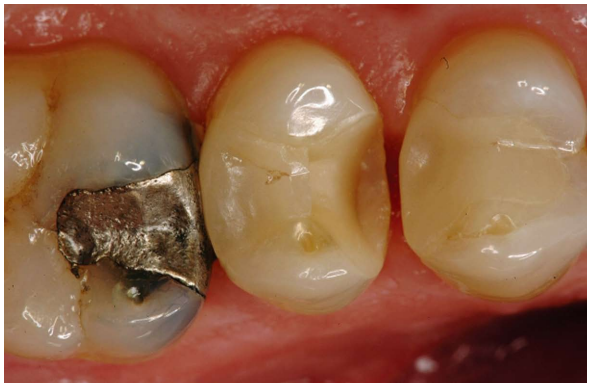

Figure 2 Fracture of a ceramic inlay in a upper first premolar.

sulfate, potassium dichromate, cobalt chloride, palladium chloride and gold sodium thiosulfate in patients with presence of metal allergy[155].

Other systemic effects (*e.g.*, mutagenic, cancerogenic or teratogenic) are more of a theoretical nature[147]. Although there is a proven amount of substance release, this does not automatically mean an inacceptable health hazard[137]. Bacsik *et al*[156] could show estrogenic effects by bisphenol-A in mice, however, it is a matter of clinical relevance when substances are directly injected into the stomach of the animals instead of investigating true release from restorations. So also here clinical studies remain the ultimate instrument for risk assessment[157]. In the course of prospective clinical studies with dental

biomaterials, the risk aspect plays a minor role. Main focus here are longevity aspects such as marginal integrity, restoration integrity, hypersensitivities, and recurrent caries. Moreover, patient numbers in dentistry *e.g.*, for studies with posterior restorations are normally in the range of 30 patients[158] which may not be of sufficient power to describe side-effects. This is clearly reflected by evaluations of side-effects with local anesthesia. Despite 0.5 million local anesthetic injections which are administered in the United States daily, the actual risks of toxicity from these local anesthetic injections remain more or less unknown[159]. Therefore, prospective clinical studies mainly concentrate on local risks such as pulp reactions, compatibility with gingiva/periodontium, irritation of the oral mucosa, or biofilm accumulation[157].

The benefit of dental biomaterials is still related to longevity. Kaplan-Meier survival curves and the associated nonparametric log rank test statistic are methods of choice for estimation of survival and therefore also failure risk[159]. This risk is appropriately reflected by annual failure rates[160,161]. Quality assessment of dental restorations is carried out according to modified USPHS critera with clinical examinations and analysis of replicas[162]. Main failure reasons are related to crucial criteria "marginal quality", "restoration integrity", "tooth integrity", and "hypersensitivities" (Figure 2)[162]. Concerning clinical success, ADA criteria of 1996 are still valid. Failure rates < 10% after 4 years are defined as acceptable.

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BRIEF ARTICLE

Effects of low intensity laser irradiation phototherapy on dental pulp constructs

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Abstract

AIM: To investigate low intensity laser irradiation phototherapy (LILIP) on the proliferation, mineralization and degradation of dental pulp constructs.

METHODS: Stem cells from human exfoliated deciduous teeth (SHED) were grown to confluence and seeded on collagen scaffolds to create dental pulp constructs. LILIP was delivered to the dental pulp constructs using an 830 nm GaAlAs laser at an output power of 20 mW. The LILIP energy density was 0.4, 0.8, 1.2, and 2.4 J/cm^2. After 8 d, the cell proliferation and degradation within the dental pulp constructs were measured using histologic criteria. After 28 d, the effect of LILIP on SHED mineralization was assessed by von Kossa staining.

RESULTS: SHED proliferation within the dental pulp constructs varied after exposure to the 0.4, 0.8, 1.2, and 2.4 J/cm^2 LILIP energy densities ($P < 0.05$). The maximum proliferation of SHED in nutrient deficient media was 218% after exposure to a 1.2 J/cm^2 LILIP energy density. SHED grown in nutrient deficient media after exposure to a 0.4, 0.8, and 1.2 J/cm^2 LILIP energy density, proliferated by 167-218% compared to the untreated (non-LILIP) control group ($P < 0.05$). SHED exposed to a 0.4, 0.8, and 1.2 J/cm^2 LILIP energy density, and grown in optimal nutritional conditions and proliferated by 147%-164% compared to the untreated (non-LILIP) control group ($P < 0.05$). The exposure of SHED to the highest LILIP energy density (2.4 J/cm^2) caused a reduction of the cell proliferation of up to 73% of the untreated (non-LILIP) control ($P < 0.05$). The amount of mineral produced by SHED increased over time up to 28 d ($P < 0.05$). The 0.8 and 1.2 J/cm^2 LILIP energy densities were the most effective at stimulating the increased the mineralization of the SHED from 150%-700% compared to untreated (non-LILIP) control over 28 d ($P < 0.05$). The degradation of dental pulp constructs was affected by LILIP ($P < 0.05$). The dental pulp constructs grown in optimal nutritional conditions exposed to a 0.8 J/cm^2 or 1.2 J/cm^2 LILIP energy density had 13% to 16% more degradation than the untreated (non-LILIP) control groups ($P < 0.05$). The other LILIP energy densities caused a 1% degradation of dental pulp constructs in optimal nutritional conditions ($P > 0.05$).

CONCLUSION: LILIP can enhance or reduce SHED proliferation, degradation and mineralization within dental pulp constructs. LILIP could promote the healing and regeneration of dental tissues.

Key words: Dental pulp cells; Proliferation; Low intensity laser; Low intensity laser irradiation phototherapy; Stem cells from human exfoliated deciduous teeth

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INTRODUCTION

Regenerative endodontics procedures are biologically based procedures that are used to replace the damaged dentin and root structures of teeth as well as cells of the pulp-dentin complex[1]. Regenerative endodontic procedures are: root canal revascularization, apexogenesis, apexification, partial pulpotomy, direct pulp capping, stem cell therapy and dental pulp constructs[1]. Endodontic regenerative procedures are widely expected to become more common in coming decades[2]. The increased usage of regenerative therapies is likely because of the discovery of dental stem cells, the use of improved treatment protocols, and the availability of new technologies[1]. The success of regenerative endodontic procedures is dependent on stimulating the proliferation and mineralization activity of stem cells from human exfoliated deciduous teeth (SHED) and other dental stem cells[3]. Previous research has demonstrated pulp healing and regeneration by adding growth factors to increase dental pulp stem cells (DPSCs) activity[4]. No previous research has investigated the possibility of using lasers to increase SHED proliferation, mineralization or degradation of scaffolds.

Lasers are beneficial for some dental treatments, such as oral surgery[5], endodontics[6], periodontology[7], and restorative dentistry[8]. Low intensity laser irradiation phototherapy (LILIP) can change cell activity[9]. LILIP has been used in the treatment of dentin hypersensitivity, gingivitis, periodontitis, and to heal oral ulcers[10,11]. In response to LILIP, fibroblast cells can increase their rate of proliferation by 300% to 600%[12]. In response to LILIP, epithelial cells cultured in a nutritionally deficient state can dramatically increase their rate of proliferation[13]. LILIP can be effective in stimulating the proliferation and mineralization activity of osteoblasts and fibroblasts[12-14]. LILIP can increase the proliferation of DPSCs, as indicated by measuring their cell mitochondrial activity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[15]. Regenerative endodontic procedures require SHED proliferation, mineralization and degradation of scaffolds if they are delivered into teeth as a dental construct[16,17] to attempt to regenerate teeth. However, the SHED responses to LILIP have not been evaluated. Consequently, there is a need to investigate the effects of LILIP on SHED proliferation, mineralization, and scaffold degradation, to identify its optimal and injurious effects, prior to its potential use as part of future regenerative endodontic procedures. The aim of this research was to investigate the effects of LILIP on the proliferation and mineralization SHED, and the degradation of dental pulp constructs.

MATERIALS AND METHODS

Cell cultures

The SHED was donated under a material transfer agreement with the National Institutes of Dental and Craniofacial Research (Bethesda, MD). Rat fibroblast L929 cells (ATTC, Manassas, VA) were used as a control treatment group cell line. The SHED were cultured in Dulbecco's modified Eagles medium (DMEM; BD Biosciences, Franklin Lakes, NJ) supplemented with 10% or 2.5% fetal bovine serum (FBS) (HyClone, Logan, UT) and 1% gentamycin and amphotericin antibiotic supplement. Cell cultures were maintained at 37 ℃ in a humidified atmosphere of 5% CO_2 with the culture media being replenished every second day. Confluent cultures of SHED were collected by trypsinization (0.25% trypsin/EDTA; Mediatech, Inc., Herndon, VA).

Preparation of three-dimensional scaffolds for cell culture

Three-dimensional collagen scaffolds (Collacote; Zimmer Dental, Carlsbad, CA) were cut into 2 mm × 2 mm sheets. Each scaffold was soaked in Hanks' balanced salt solution (HBSS; Cellgro, Herndon, VA) and stored at 4 ℃. Before use, the HBSS was replaced by culture medium. The scaffolds were incubated in DMEM at 37 ℃ for 30 min before application of the cells to equalize culture conditions and temperature between the scaffolds and cells.

Creation of dental pulp constructs

The SHED were added to the scaffolds in fresh aliquots; each scaffold was seeded with a million ($\times 10^6$) cells to create dental pulp constructs[16,17]. The L929 ($\times 10^6$) cells were also applied to the scaffolds as a control treatment. The scaffolds were maintained in 6-well culture plates (BD Biosciences, Franklin Lakes, NY) containing 5 mL of culture media. The DMEM culture media was removed and replenished every 2 d. After 8 d of cell culture, the dental pulp constructs were transferred to 96-well plates.

Laser irradiation of dental pulp constructs

Laser irradiation was delivered with Gallium-Aluminum-Arsenide (GaAlAs) laser (Asah Medico Uni-Laser, Hvidovre, Denmark). The irradiations were performed in contact with the plate base using the punctual irradiation mode in a 0.252 cm^2 area[15]. The 830 nm laser was applied with output power setting of 20 mW. The laser device was calibrated using laser power meter (Model OPM-572; Sanwa, Tokyo, Japan). The clear base of a well from 96-well plate was separated and the laser power output was measured after the laser passed through the base to determine the exact energy density on the cells. This measurement was repeated three times and the aver-

age was calculated. The energy density was applied using 6, 12, 18, and 36 s of irradiation time. The energy density was calculated using the following formula: energy density (J/cm^2) = [power (W) × time (s)]/area (cm^2).

Vital staining of SHED in dental pulp constructs

During the cell culture of the dental pulp constructs, 0.0016% neutral red dye (JT Baker, Phillipsburg, NJ) cell viability marker was added to the DMEM in order to stain the vital cells dark red[1]. The SHED and L929s were cultured for 8 d on the collagen scaffolds, with 10 culture replicates for each of the constructs treatments. For each cell type, the experimental groups were: lased 6 s with 0.4 J/cm^2, lased 12 s with 0.8 J/cm^2, lased 18 s with 1.2 J/cm^2, and lased 36 s with 2.4 J/cm^2. The control for this investigation was including constructs without irradiation.

Histology of dental pulp constructs

The dental pulp constructs were removed from cell culture and fixed by submerging them in a 10% neutral-buffered formalin (BDH Chemicals, Poole, United Kingdom) solution for 24 h. All the tissue constructs were then dehydrated in a graded series of alcohols from 70% to 100%. The constructs were then embedded in paraffin wax blocks and cut into serial histologic sections of 5-μm thickness using a microtome. The histology sections were then mounted onto glass slides and covered with a cover slip using adhesive.

Pathohistometric analysis of tissue constructs

The numbers of stained neutral red SHED were counted as the number of vital metabolically active cells within each of the dental pulp constructs[16]. The cells were counted using pathohistometric analysis, the numbers of cells per microscope field with 5 random microscope fields being counted per specimen using a light microscope (Vista vision, VWR Scientific, West Chester, PA) at × 200 magnifications with a reticule[18]. Construct degradation was measured as an area of scaffold that no longer existed using a light microscope at × 200 magnifications with a reticule.

Assessment of mineralization capacity of SHED

SHED and L929s were cultured and treated with LILIP using the same energy density and 6, 12, 18, and 36 s of irradiation time which was described previously. The SHED and L929s cells were incubated with DMEM mineralization induction media, supplemented with 10 mmol/L sodium β-glycerophosphate (Sigma, St. Louis, MO, United States), for 28 d. The mineralization was assessed by a von Kossa staining (Diagnostic BioSystems, Pleasanton, CA)[19]. The mineralized cultures were fixed with 10% buffered formalin for 30 min. Subsequently, they were washed and stained with von Kossa silver and exposed to ultraviolet light for 30 min. Then cells were treated with 5% sodium thiosulfate for 2 min and washed again. The mineralization capacity of each cell line was determined and compared by measuring the density of mineral nodules formed in each cell type using the tagged image format (tif) image for manipulation in Adobe Photoshop (Adobe Systems, San Jose, CA). Mineralization was measured in three random areas of each specimen.

Statistical analysis

The data were analyzed using an analysis of variance statistical test, followed by Scheffe's multiple comparison tests between treatment groups (Statview, SAS Institute Inc., Cary, NC). A P value of $P < 0.05$ was considered statistically significant.

RESULTS

LILIP output power and energy density

The output power setting of 20 mW of the GaAlAs laser was measured by the laser energy meter as 16.83 mW reaching the SHED through the plastic base of the 6 well plates. After applying the LILIP energy density for 6, 12, 18, and 36 s, the energy density was calculated using a formula to be 0.4, 0.8, 1.2, and 2.4 J/cm^2.

Nutritionally deficient FBS concentration for SHED

Prior to experimentation a pilot study of the effects FBS concentrations (10%-1%) within the DMEM culture media found that a 2.5% FBS concentration, was the minimal concentration of FBS necessary to avoid SHED death and reduced cell proliferation. The 2.5% FBS concentration met the criteria[12,13,15] to be the conditions of SHED nutritional deficit, and the 10% FBS concentration was the optimal nutritional condition.

SHED proliferation following LILIP

A pilot study revealed that the maximum SHED responses were seen 8 d or more following exposure to LILIP, consequently the SHED responses in this present study were measured 8 d after exposure to LILIP. SHED proliferation within the dental pulp constructs varied after exposure to the 0.4, 0.8, 1.2, and 2.4 J/cm^2 LILIP energy densities ($P < 0.05$). The maximum proliferation of SHED in nutrient deficient FBS media was 218% after exposure to a 1.2 J/cm^2 LILIP energy density. SHED grown in nutritional deficient media after exposure to a 0.4, 0.8, and 1.2 J/cm^2 LILIP energy density, proliferated by 167%-218% compared to the untreated (non-LILIP) control group ($P < 0.05$). SHED exposed to a 0.4, 0.8, and 1.2 J/cm^2 LILIP energy density, and grown in optimal nutritional conditions and proliferated by 147%-164% compared to the untreated (non-LILIP) control group ($P < 0.05$). The exposure of SHED to the highest LILIP energy density (2.4 J/cm^2) caused a reduction of the cell proliferation of up to 73% of the untreated (non-LILIP) control ($P < 0.05$). The nutrient deficient (2.5%) FBS culture media and optimal (10%) FBS culture media had little effect on the loss of SHED proliferation following exposure to the highest (2.4 J/cm^2) LILIP energy density ($P > 0.05$). The loss of proliferation (62%)

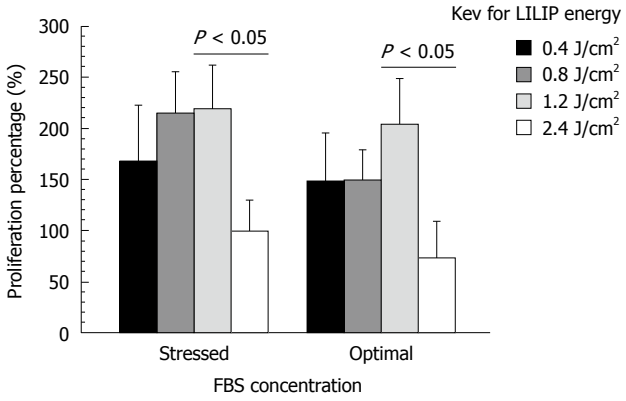

Figure 1 Bar chart of the stem cells from human exfoliated deciduous teeth proliferation within the dental pulp constructs with different low intensity laser irradiation phototherapy energy densities and different fetal bovine serum concentrations. LILIP: Low intensity laser irradiation phototherapy; FBS: Fetal bovine serum.

of L929 was less than the loss of proliferation (73%) of SHED after exposure to the highest (2.4 J/cm^2) LILIP energy density (Figure 1).

SHED mineralization following LILIP

The amount of mineral produced by SHED varied 28 d after exposure to 0.4, 0.8, 1.2, and 2.4 J/cm^2 LILIP energy densities ($P < 0.05$). SHED produced (106%-255%) more mineral than L929 cells ($P < 0.05$). The amount of mineral produced by SHED increased over time up to 28 d ($P < 0.05$). The 0.8 and 1.2 J/cm^2 LILIP energy densities were the most effective at stimulating the SHED to produce minerals over 28 d ($P < 0.05$) (Figure 2). The 0.8 and 1.2 J/cm^2 LILIP energy densities increased the mineralization of the SHED from 150%-700% compared to untreated (non-LILIP) control SHED.

SHED degradation of constructs following LILIP

The degradation of dental pulp constructs was affected by LILIP ($P < 0.05$). The dental pulp constructs grown in optimal nutritional conditions exposed to a 0.8 J/cm^2 or 1.2 J/cm^2 LILIP energy density had 13% to 16% more degradation than the untreated (non-LILIP) control groups ($P < 0.05$). The other LILIP energy densities were less effective at causing the degradation of dental pulp constructs (Figure 3).

DISCUSSION

Regenerative endodontic procedures are successful because SHED and other dental stem cells can regenerate dentin and dental-pulp tissues[20]. The stimulation of SHED to increase proliferation, mineralization and scaffold degradation can be important to ensure that endodontic healing is quick and effective. This is the first investigation of using LILIP to control SHED proliferation, mineralization and construct degradation. This is also the first investigation to determine the optimal and injurious ranges of LILIP energy densities to enhance and inhibit the activity of a dental stem cell line.

The GaAlAs laser was used with an output power setting of 20 mW, but the laser energy meter measured only 16.83 mW reaching the SHED through the plastic base of the 96 well plates. The loss of 15.9% of the laser energy was factored into the energy densities (0.4, 0.8, 1.2, and 2.4 J/cm^2) of this present study. A limiting factor in some previous laser studies[15,21-23] is that the energy densities reaching the cells were not measured by a meter, and so it is not clear what actual energy density was used. In this study, the LILIP parameters (wavelength, power output, irradiated area), were kept constant, except the irradiation times (6, 12, 18, and 36 s, and their corresponding energy densities (0.4, 0.8, 1.2, and 2.4 J/cm^2). The GaAlAs laser wavelength was 830 nm, which is ideal for LILIP[22,23], but low energy compared to other laser types.

The effect of LILIP has been studied on several cell types[12-14]. A previous study found that LILIP can increase the proliferation of DPSCs, by measuring their cell mitochondrial activity using the MTT assay[15]. However, it is not clear what the precise change in the rate of DPSCs proliferation was, or if the 20 mW and 6 s of LILIP energy density[15] was the most optimal, since no other energy densities were investigated, or if DPSCs and SHED used in this present study, share similar responses to LILIP.

The present study discovered that the LILIP energy density could enhance or reduce SHED proliferation and degradation within dental pulp constructs. SHED proliferation increased following exposure to 0.4, 0.8, and 1.2 J/cm^2 LILIP energy densities following culture in both the optimum and nutrient deficient FBS conditions. After prolonged exposure to 2.4 J/cm^2 LILIP energy density, the proliferation of SHED was inhibited. The results indicate that a 1.2 J/cm^2 LILIP energy density is optimal to enhance SHED proliferation. This is consistent with previous research demonstrating that LILIP can stimulate cell proliferation in a narrow energy range, and with low energy density[24]. Excessive LILIP energy densities can inhibit cell proliferation[12,23]. The range of energy densities in this current study which could enhance or reduce SHED activity is in accordance with the Arndt-Shultz Law[25]. The Arndt-Shultz Law predicts that a small amount of laser or other source of energy will increase physiological activity, and that a larger amount of the energy will kill cells[12,23]. In this study, the energy stimulation range was between 0.4-1.2 J/cm^2 and the energy inhibition occurred at a 2.4 J/cm^2 energy density.

SHED are beneficial for regenerative endodontics because they can differentiate into mineralizing cells which can regenerate teeth[26]. In the von Kossa staining part of the present study, the SHED treated with mineralization induction media, we observed to deposit substantial amounts of mineral nodules (black color). The formation of mineral nodules suggests that the SHED differentiated into an odontoblast-like type of cell[27]. The SHED had a greater capacity for mineralization than the control L929 cultures. All the cell cultures showed ascending

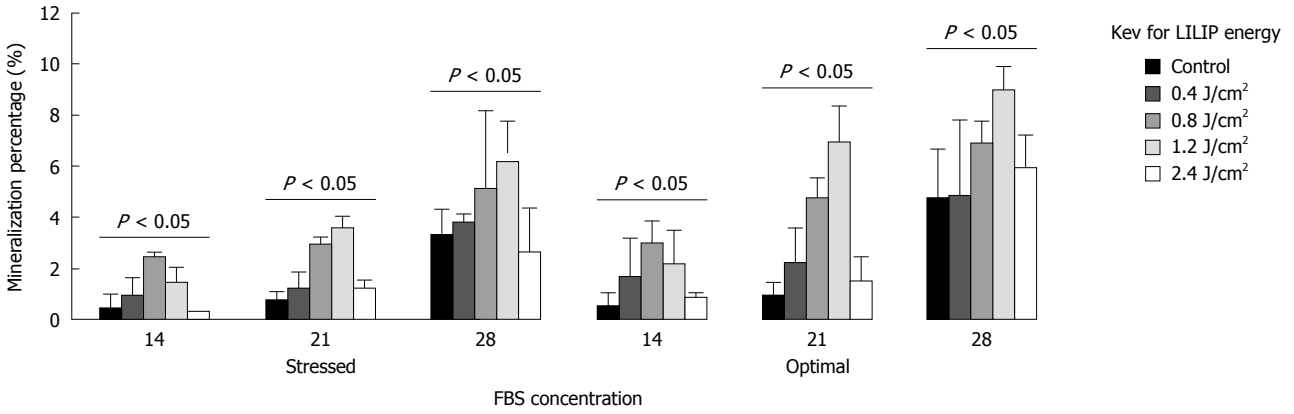

Figure 2 Bar chart of the mineralization percentages of stem cells from human exfoliated deciduous teeth cultures with different low intensity laser irradiation phototherapy energy densities and different fetal bovine serum concentrations. LILIP: Low intensity laser irradiation phototherapy; FBS: Fetal bovine serum.

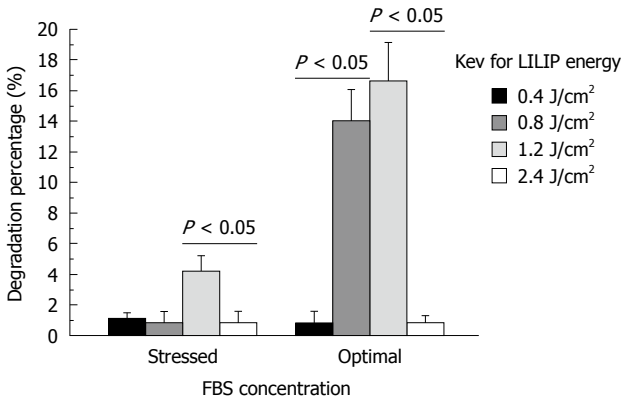

Figure 3 Bar chart of the degradation within the dental pulp constructs with different low intensity laser irradiation phototherapy energy densities and different fetal bovine serum concentrations. LILIP: Low intensity laser irradiation phototherapy; FBS: Fetal bovine serum.

mineralization percentages from 14-28 d regardless the cell line or the FBS concentrations. The cultures supplemented with optimal FBS concentration showed higher percentages of mineralization than the stressed cultures. This indicates that nutrient deficient SHED has a lower capacity for mineralization, suggesting that a nutrient deficit can reduce cell mineralization activity.

The molecular mechanism whereby LILIP can increase cell activity is reported to be its ability to increase the concentration of calcium in the cytoplasm from the mitochondria[9,28,29]. Consequently, the calcium transported into the cytoplasm can increase the rate of cell mitosis and improve cell proliferation. Further research is needed to identify how the molecular mechanisms of cells can be targeted to cause them to proliferate, differentiate and mineralize, as an alternative to the traditional use of growth factors for this purpose[4].

In conclusion, a 1.2 J/cm^2 energy density of LILIP enhances SHED proliferation, dental pulp construct degradation, and mineralization. These results are significant because SHED and other dental cell proliferation, dental pulp construct degradation, and mineralization are needed to make regenerative endodontics quick and effective. Future clinical research is needed to more completely identify the regeneration benefits of using LILIP, such as following the accidental exposure of the dental pulp, Cvek pulpotomy, tooth revascularization and regeneration.

COMMENTS

Background

This is the first article to describe using low intensity laser irradiation phototherapy (LILIP) to control stem cells from human exfoliated deciduous teeth (SHED) proliferation, mineralization and construct degradation. This is also the first article to determine the optimal and injurious ranges of LILIP energy densities to enhance and inhibit the activity of a dental stem cell line.

Research frontiers

A hotspot of dental research is to develop new therapies which can promote dental tissue healing and regeneration. LILIP could be used to activate SHED and potentially other stem cells to regenerate missing dental tissues.

Innovations and breakthroughs

At the current time, lasers are most often used to cut soft dental tissues. LILIP is a new type of laser therapy. LILIP could be used more frequently in future dental practice to regenerate missing tissues for patients.

Applications

Future clinical research is needed to more completely identify the applications of using LILIP, such as to promote the healing of the exposed dental pulp and in conjunction with Cvek pulpotomy, tooth revascularization and regeneration procedures.

Peer review

The reviewers found the article to be innovative and interesting. The article is significant because it is the first article to evaluate the effects of LILIP on SHED within dental pulp constructs.

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BRIEF ARTICLE

Ozone action on *Streptococcus mutans* and *Lactobacillus fermentum*: A pilot study

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Abstract

AIM: To study the effectiveness of ozone in the elimination of cariogenic bacteria, followed with fluoride supplements.

METHODS: Sixty extracted teeth free of caries were used, and five groups were constituted. In Group Ⅰ, the teeth were immersed in artificial saliva. In Group Ⅱ, the teeth were inoculated with *Streptococcus mutans* (*S. mutans*) and immersed in artificial saliva. In Group Ⅲ the teeth were inoculated with *Lactobaccilus fermentum* (*L. fermentum*) and immersed in artificial saliva. In Group Ⅳ the teeth were inoculated with *S. mutans* and *L. fermentum* and immersed in artificial saliva and the teeth in Group Ⅴ were inoculated with *S. mutans* and *L. fermentum*, and were subjected to the application of ozone and to the action of a fluoride mineralizing gel. DIAGNOdent was used to evaluate the caries of the teeth 3 wk after inoculation of bacteria and after that the teeth of Group Ⅴ were subjected to the application of ozone during 60 s, by HealOzone. After the application of ozone, products of the remineralization kit supplied by the manufacturer were applied daily, during 30 d. At the end samples were collected for analysis and evaluation of bacterial activity by polymerase chain reaction.

RESULTS: Regarding the value of caries, obtained *via* DIAGNOdent, in the initial measurement the groups are homogeneous (P = 0.730). There was an increase in DIAGNOdent values, presenting statistical significant difference regarding the initial measurement in all groups (P < 0.001), except in group Ⅰ - only artificial saliva - which shows that the artificial carie model was effective. Comparing the initial and final measurements for each of the 60 teeth, it can be observed that in 9 teeth (15.0%) there was a decrease in values between the two measurements, one (1.7%) retained the same values in the two measurements and in the remaining 50 cases (83.3%) there was increase in values between the initial and final measurements. It should also be noted that in the teeth inoculated with *S. mutans* + *L. fermentum*, there was an increase of the values in 100% of cases, and in all groups except the group with artificial saliva, there is a more frequent increase in the values. In group Ⅴ, subject to the application of ozone, bacterial DNA was not detected, in group Ⅳ, bacterial DNA was detected.

CONCLUSION: Ozone was effective in the elimination of the study bacteria.

Key words: Dental caries; Cariogenic agents; *Lactobacilus fermentum*; *Streptococcus mutans*; Ozone

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INTRODUCTION

Currently dental caries can be defined as a multifactorial and infectious disease, characterized by a localized hard tissue demineralization of tooth, resulting from acidic products, through bacterial fermentation of carbohydrates[1,2].

Clarke[3] (1924) identified *Streptococcus mutans* (*S. mutans*) in carious lesions and 36 years later Fitzgerald and Keyes found that these bacteria were capable of inducing caries in hamsters. A number of studies in humans have shown that caries are a confirmation of bacterial infection, primarily mediated by *S. mutans*, which is transmited through the saliva within the family unit. *Lactobacillus fermentum* (*L. fermentum*) is also associated with the caries process, but is not responsible for it, since it does not have the capacity for the adherence like *S.* mutans[3].

Until today no single factor was found as a caries inducer, instead several factors are proposed such as: microorganisms, diet, teeth and saliva, resulting in the metabolic activity of bacteria that will ferment diet carbohydrates to produce lactic acid[1,2,4]. Metabolic activity leads to changes of pH on the interface of the tooth surface and bacterial deposits, producing an imbalance between the enamel and the fluid of the plaque, causing the loss of tooth mineral when pH decreases, or the mineral gain when the pH increases[1]. The cumulative result of these processes can be demineralization or remineralization, with mineral loss, leading to dissolution of the dental tissues and the formation of caries[1].

In strategies for management of caries, emphasis has been given to preventive treatments and remineralizing procedures in the early detected stages[1,5]. In the presence of caries we have various treatment options. The decision in cavitated teeth becomes simpler, since the treatment choice is conventional drilling and filling[5]. In the lesions that are not cavitated, the decisions become more complex, two types of approach can be selected: invasive, assuming that the lesion is active and will progress; or a conservative approach, in order to stop the progression of caries. The latter approach is where emphasis has been placed, leading to more research in this area involving fluoride products and the remineralization process[6]. These approaches can be divided into selective invasive interventions and non-invasive interventions, in which ozone is included[7]. The non-invasive interventions focus on caries prevention and the preservation of demineralized enamel and dentin, but without cavitation. Under these conditions "restitutio ad integrum" is thought to be possible, by removing bacteria and products of their metabolism and allowing the remineralizing process using fluoride[7].

The antimicrobial effects of ozone have been known for many years[5]. The direct application of this gas on coronary or root surfaces seems to have a sterilization effect[8]. It is mentioned that it is able to slow, stop, or reverse the carious process[7]. It is believed that the ozone is also useful in reducing the microbial flora in cavitated lesions, before proceeding to filling[7]. Although ozone is not a radical, it is the third most potent oxidant[9]. Due to its oxidative power, it induces the destruction of the cell walls and membranes, and cytoplasm of bacteria, fungi and certain viruses[10,11]. The use of ozone in dentistry has been advocated for the sterilization of cavities, root canal, periodontal pocket and herpetic lesions[9]. However, its major indication is in the treatment of caries[4,5]. The ozone procedure involves the application of ozone gas, the use of remineralizing agents and an oral hygiene kit to give to the patient, in order to promote the process of remineralization.

The present work aims to assess the effectiveness of ozone in the elimination of cariogenic bacteria.

MATERIALS AND METHODS

Teeth preparation

Sixty extracted premolars and molars without caries were selected and stored in a saline solution. The extracted teeth were set in acrylic blocks and numbered. Before using the DIAGNOdent® pen 2190 (KAVO GmbH, Biberach, Germany) to evaluate the caries, the teeth were cleaned, dried and selected according the value obtained with DIAGNOdent. An occlusal recess of small size (0.08 mm wide, 1 mm deep and 3 mm in length) was carried out, with a 008 cylindrical diamond drill, placed in turbine with cooling air/water.

Artificial saliva

Artificial saliva solution was used and prepared based on a modification of the basal medium mucin (BMM) medium with modified Shellis artificial saliva, whose composition is described in the Table 1. The bacteria used were *S. mutans* (ATCC reference: 25 175) and *L. fermentum* (ATCC reference: 14 932). These bacteria were grown and maintained according to the instructions in the ATCC (American Type, Culture and Collection). For inoculation of the samples, bacteria were prepared in phosphate buffered solution (PBS) suspensions. This was subsequently diluted to the final value of 10^5 colony forming units for each bacteria of saliva used for this study. Artificial saliva was supplemented with 2% glucose (20 g/L).

Constitution of groups

Five groups were formed: Group Ⅰ - 10 teeth, immersed in artificial saliva; Group Ⅱ - 10 teeth, inoculated with *L. fermentum* with a bacterial load of 10^5 and immersed in artificial saliva; Group Ⅲ - 10 teeth, inoculated with *S. mutans* with a bacterial load of 10^5 and immersed in artificial saliva; Group Ⅳ- 10 teeth, inoculated with *L. fermentum* and *S. mutans* with a total bacterial load of 2×10^5 and immersed in artificial saliva; Group Ⅴ - 20 teeth inoculated with *L. fermentum* and *S. mutans* with a total bacterial load

Table 1 Composition of artificial saliva, modification of basal medium mucin Medium with modified artificial Shellis saliva

	g/L
Yeast extract	5
Proteose peptone	10
Potassium chloride (KCl; 74.5 g/mol)	1.2
Sodium hydrogen carbonate (NaHCO3; 84 g/L)	0.6
Potassium thiocyanate (KSCN; 97.18 g/mol)	0.23
Sodium Di-hydrogen phosphate (Na_2HPO 4.12 H_2O; 358 g/mol)	0.9

of 2×10^5, subject to the application of ozone followed by fluoride remineralizing gel (supplied by the manufacturer of HealOzone, with sodium fluoride 0, 24% w/w) and immersed in artificial saliva.

All teeth during the experimental protocol were kept in an incubator at 37 ℃ with permanent stirring at 150 r/min. All procedures performed during the experimental protocol were performed in a laminar flow chamber. The teeth were immersed in artificial saliva and inoculated in accordance with the specifications for each experimental group. DIAGNOdent was used to evaluate the teeth 3 wk after inoculation, as well as the pH, using a pH measuring tape (pH 1-10 Universalindikator Merck).

Ozone application

The application of Ozone was made with HealOzone® in group V (KAVO GmbH, Biberach, Germany) 3 wk after bacterial inoculation. Before ozone application, the teeth were removed from the medium and washed with a sterile saline solution, and ozone was applied for 60 s. For ozone application a silicone dome was used, which was selected according to the size of each tooth, and changed between each tooth. After application of the ozone a reducing solution was applied, supplied by the manufacturer, for 60 s. Then the teeth were immersed in fresh medium again. Glucose at 0.05% was added to the new medium, also composed of artificial saliva. The bottles were flushed with sterile saline solution before the new medium was inserted. 30 d later, the teeth were brushed with a tooth brush and tooth paste provided by the manufacturer of HealOzone. At the end of brushing a fluoride spray was applied, also supplied by the manufacturer, as a remineralization protocol once a day.

Sample preparation

Seven weeks after inoculation, a collection of Groups Ⅳ and V was made, prepared by excavation of cavities, with a dentin sterile excavator. The samples were stored in PBS and 25% glycerol. The extraction of total DNA from bacteria was performed by the method of proteinase K. Extraction was performed using the protocol of bacteria Lysis Buffer from Roche. After the extraction, the DNA was quantified.

Statistical analysis

Statistical analysis of results shows the mean values and standard deviation. Comparison between groups was performed using Kruskal-Wallis test with multiple comparisons and adjusted in each group, by the Wilcoxon test. Comparisons between the initial and final measurements in each tooth and their relation with group was performed using a χ^2 test with simulation by the Monte Carlo method ($P < 0.001$). All data analysis was performed by SPSS, version 19, at a significance level of 5%.

RESULTS

Regarding the value of caries, obtained with DIAGNOdent, in the initial measurement groups are homogeneous ($P = 0.730$) but the second measuring statistical difference exist between groups, the differences being detected between groups: group I and group V ($P < 0.001$), group I and group Ⅳ ($P = 0.014$), group Ⅳ and group V ($P = 0.036$).

There was an increase in DIAGNOdent values, presenting statistical significant difference regarding the initial measurement in all groups, except in the group I , only artificial saliva, see Table 2.

Comparing the initial and final measurements for each of the 60 teeth, it can be observed that in 9 teeth (15.0%) there was a decrease in values between the two measurements, one (1.7%) retained the same values in the two measurements and in the remaining 50 cases (83.3%) there was an increase in values between the initial and final measurements. Although changes are associated with the group, a decrease of the values can be expected only in the group which used artificial saliva and an increase in teeth with *S. mutans* or *S. mutans* + *L. fermentum*. The maintenance of values between measurements occurred in the *L. fermentum* group. It should also be noted that in the teeth inoculated with *S. mutans* + *L. fermentum* there was an increase of the values in 100% of cases, and in all groups except the group with artificial saliva there is a more frequent increase in the values (Table 3).

In group V, subject to the application of ozone, bacterial DNA was not detected. In group Ⅳ, bacterial DNA was detected, polymerase chain reaction amplification curves, for some teeth of group Ⅳ are presented in Figure 1.

DISCUSSION

At the beginning of the experimental work, all teeth were free from caries. The highest values accepted, quantified by DIAGNOdent® pen 2190, were 12. According to the recommendations of KaVo, this corresponds to the threshold for considering the tooth tissue normal and healthy. The diagnosis of caries lesions is made after visual and tactile examination of tooth surfaces, however it may be necessary the use auxiliary diagnostic tests[1]. A tool developed for the diagnosis and quantification of caries is DIAGNOdent, which is based on tissue fluorescence induced by laser light. In interpreting the values obtained, it is necessary to take into consideration that there may be false positives. These can occur in the fol-

Table 2 Results of DIAGNOdent, expressed as mean ± SE, quartiles and range of variation

Material	Med	*n*	Mean	Structural equation modeling	Min	Max	P25	P50	P75	Med1 *vs* Med2
Artificial Saliva	1	10	6.10	0.795	2	11	4.75	6.00	7.50	0.759
	2	10	8.20	4.54	0	45	0.00	1.00	14.75	
L. fermentum	1	10	5.40	0.792	2	10	3.00	5.50	7.25	0.011
	2	10	19.40	6.31	2	63	7.00	9.50	27.50	
S. mutans	1	10	5.60	0.859	2	9	2.00	6.50	8.00	0.011
	2	10	30.00	10.33	0	99	9.25	15.50	47.00	
S. mutans + *L. fermentum*	1	10	6.00	0.978	2	12	4.50	5.00	8.50	0.005
	2	10	31.90	3.29	16	47	25.00	29.50	43.75	
S. mutans + *L. fermentum* ozone + remin	1	20	5.15	0.782	1	12	2.25	4.00	8.75	< 0.001
	2	20	45.95	5.90	20	99	24.50	37.00	68.50	
Comparisons between 5 groups	1	$P = 0.730$								
	2	$P < 0.001$								

S. mutans: *Streptococcus mutans*; *L. fermentum*: *Lactobacillus fermentum*.

Table 3 Measurements difference for each of 60 teeth

			Group					Total
			Artificial saliva	*L. fermentum*	*S. mutans*	*S. mutans* + *L. fermentum*	*S. mutans* + *L. fermentum* + ozone	
Difference	Decreased	*n* (%)	7	1	1	0	0	9
		Residue	5.5	-0.5	-0.5	-1.5	-3.0	
	Maintenance	*n* (%)	0	1	0	0	0	1
		Residue	-0.2	0.8	-0.2	-0.2	-0.3	
	Increased	*n* (%)	3	8	9	10	20	50
		Residue	-5.3	-0.3	0.7	1.7	3.3	
Total			10	10	10	10	20	60

S. mutans: *Streptococcus mutans*; *L. fermentum*: *Lactobacillus fermentum*.

lowing situations: teeth with plaque; fillings with fluorescent composites; proximity of the pulp; food waste; prophylactic pastes; remineralized cavities; increased natural fluorescence and patients exposed to radiation.

Three weeks after bacterial inoculation, the presence of caries was evaluated in all groups, using DIAGNOdent® pen 2190. Some authors claim that the diagnostic technologies such ECM (Electric Caries Meter) and DIAGNOdent, perform better in the early detection of caries in occlusal surfaces, compared with visual examination[12]. Goel *et al*[13] in 2009 conducted an *in vivo* study that compares the efficacy of DIAGNOdent with conventional methods (visual, tactile and bitewings radiographs). The authors concluded that DIAGNOdent showed high precision compared to conventional methods for the detection of enamel caries. However, when the cavities reach dentine, although precision is high, it is similar to other diagnostic methods. The DIAGNOdent is the diagnostic method chosen for this work since it allows the quantification of dental caries, has good accuracy, reproducibility, sensitivity and validity[12,13].

Plaque in humans is a complex biofilm comprising hundreds of microbiological species[14]. The culture plaque using the BMM medium, exhibits similar growth rates behaviour to the natural. Oral fluids are the major source of nutrients to bacterial plaque[14]. Several studies simulating these oral fluids *in vitro* contained as main components mucin, yeast extract and/or peptones. One of these formulations is the BMM medium, which has been widely used for *in vitro* studies of oral bacteria[14]. The presence of yeast extract and peptones contributes to the presence of a variable concentration of peptides, vitamins and ions in the medium[14]. Shellis in 1978 developed an artificial saliva, chemically defined, containing various ions, amino acids, vitamins, growth factors and bovine origin mucin[14]. This has as its major components potassium chloride, sodium chloride, sodium hydrogencarbonate and potassium thiocyanate[14].

The artificial saliva used in this experimental study consisted of a modification of BMM medium, with modified Shellis saliva, which since the system used was a simple one, only comprises two bacteria. To allow a greater bacterial growth prior to inoculation of bacteria on teeth, the artificial saliva was supplemented with 2% glucose, to allowing the formation of lactic acid by fermentation.

After the appearance of the tooth caries and application of ozone, the teeth were immersed in new medium, the same as used earlier, but without glucose supplementation, since the carious lesion was established and the concentration of glucose in yeast extract must correspond to 0.05% and the concentration of glucose in saliva in an adult is 0.01%[15].

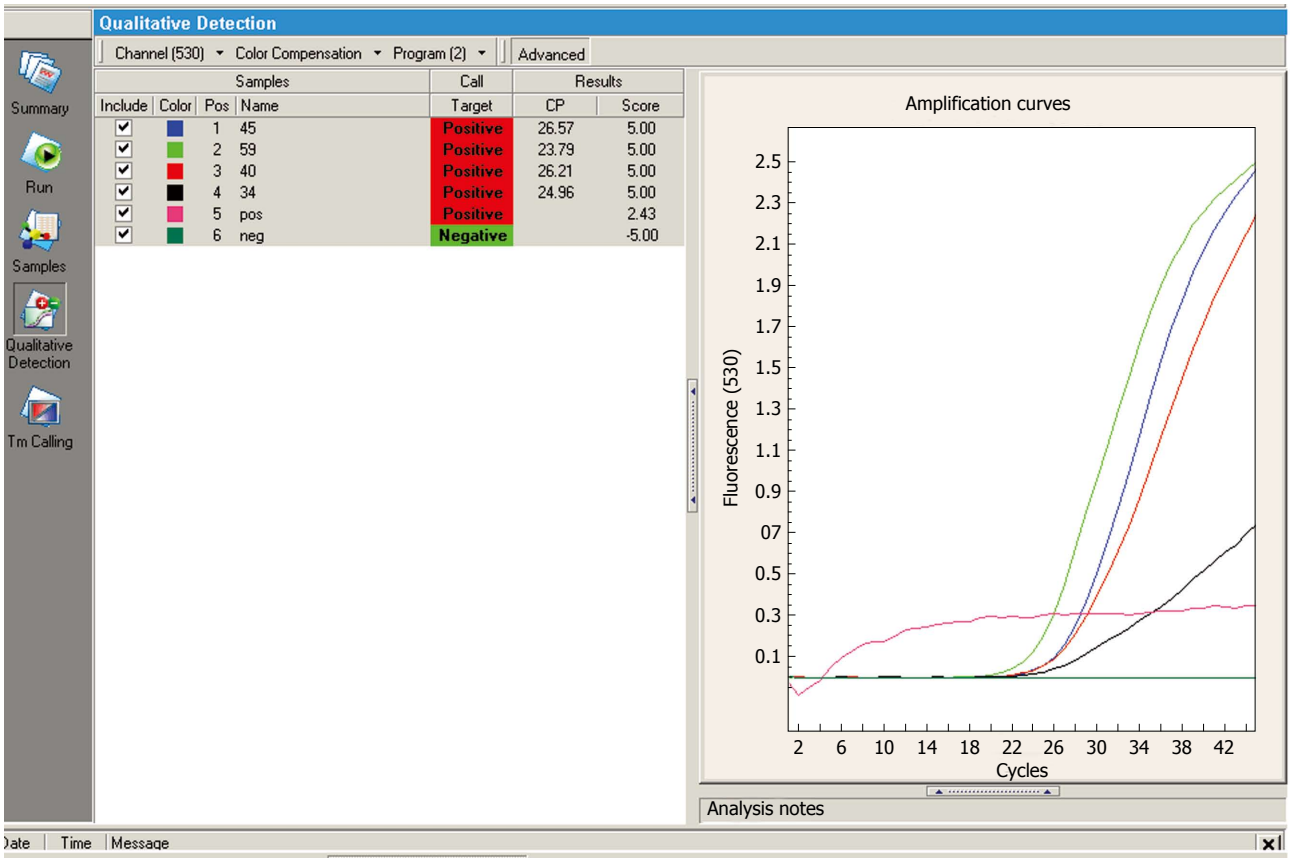

Figure 1 Polymerase chain reaction amplification curves of real-time for the group Ⅳ, inoculated with *Streptococcus mutans* and *Lactobacillus fermentum*.

In order to promote bacterial retention on site and to allow faster development of the caries process, we performed a cavity of small dimensions on the occlusal surface as the occlusal surfaces are particularly susceptible to caries[1].

The antimicrobial effect of ozone has been shown by several studies[11,16]. However there are studies that show conflicting results, such as those done by Baysan *et al*[17] in 2007, which demonstrated that the application of ozone in carious lesions of dentin did not significantly reduce the number of bacteria detected in infected dentin in non-cavitated caries. Castillo *et al*[11] in 2008 conducted an *in vitro* study, which used 41 strains of *S. mutans* including native strains obtained from the saliva of 27 children, which after being cultured, were placed in eppendorf. The ozone was applied to the eppendorf and the results show that after application of ozone for 40 s, no bacteria were viable. Polydorou *et al*[16] in 2006, compared the antibacterial effect of ozone with two dentin adhesive systems. The adhesive systems showed significant antibacterial activity. However, application of ozone has proved a promising in eliminating residual microorganisms in deep cavities and potentially increases the clinical success of fillings. Knight *et al*[18] in 2008 conducted an *in vitro* study to determine the effects of ozone application in dentin before the formation of a biofilm. The study showed that the ozone application prevents the formation of a biofilm of *S. mutans* and Lactobacillus acidophilus for a period of 4 wk.

Studies conducted to evaluate the efficacy of ozone in carious lesions in pits and fissures and roots already have a good level of scientific evidence, since they are randomized. However, there are authors who raise questions about the evaluation method, which is in most cases performed by DIAGNOdent, and as to whether the participants were aware of the treatment administered to them (only the study by Holmes is double-blind) and that many of the studies were conducted by the team members from the main precursor of this therapy, Edward Lynch[10]. Systematic reviews on the subject are unanimous about the fact that more scientific evidence is needed that this therapy can be accepted as an alternative therapeutic approach to early detected caries[5,10].

From the study it can be concluded that the caries induction protocol employed in this study was effective in the development of caries lesions. The application of ozone through HealOzone for 60 s, at a concentration of 2100 ppm was effective in eliminating caries bacteria. No bacterial DNA was detected after the application of ozone, followed by the daily application of the remineralizing products for 30 d. The treatment with ozone is advantageous in minimally invasive dentistry, it maintains the healthy tissue, and does not require anaesthesia, and with a simple and non-time-consuming process presents satisfactory results. More evidence is still needed before the cost-benefit ratio can be assessed.

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COMMENTS

Background

Carious lesions occur due to acid dissolution of the enamel and/or dentin as a result of the metabolism of specific microorganisms, the main ones being *Streptococcus mutans* (*S. mutans*) and *Lactobacillus fermentum* (*L. fermentum*). Ozone is a powerful oxidant that has the ability to eliminate 99% of bacteria, fungi and viruses. Once the bacteria is eliminated, the remineralization may occurs in the treated area.

Research frontiers

The bacterias *S. mutans* and *L. fermentum* are effective in producing tooth decay immersed in artificial saliva, and ozone was able to eliminate them. In this study the authors shows the capability of ozone in their elimination.

Innovations and breakthroughs

This is the first study to demonstrated the capability of bacteria elimination by the ozone, by polymerase chain reaction. Demonstrating that the ozone is highly effective. This study by demonstrated the bacteria elimination, shows that the ozone provides conditions for the remineralization.

Applications

Ozone therapy is advantageous in minimally invasive dentistry, because it maintains healthy tissue, does not require anesthesia and with a simple and non-time-consuming procedure gives satisfactory results.

Terminology

The carie occurs due to acid dissolution of the enamel and/or dentin as a result of the metabolism of specific microorganisms, the main are *S. mutans* and *L. fermentum*. Ozone is oxidant that has the ability to eliminate bacteria, fungi and viruses.

Peer review

The manuscript is good to add some information on the antibacterial effect of ozone in dental caries.

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BRIEF ARTICLE

MMP-8 analysis in gingival crevicular fluid using ELISA and novel chair-side test

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Abstract

AIM: To validate accuracy of a novel chair-side test for matrix metalloproteinase (MMP)-8 as compared to enzyme-linked immunosorbent assay (ELISA) in Periodontal health and disease.

METHODS: Gingival crevicular fluid was collected from 150 subjects, Group 1 (healthy) - 50 subjects, Group 2 (gingivitis) - 50 subjects and Group 3 (chronic periodontitis) - 50 subjects. A chair-side test strip was indigenously prepared using polyclonal antibodies (principle of immunochromatography) to detect the MMP-8 levels. The detection accuracy (sensitivity and specificity) of the MMP-8 levels by chair-side test kit were compared with ELISA at baseline and 3 mo after scaling and root planing among the study population.

RESULTS: The novel chair side test detected MMP-8 levels in accordance with ELISA which at baseline were higher in Group 2 and Group 3 as compared to controls ($P < 0.05$), and these enzyme levels decreased after therapy ($P < 0.05$). The chair-side test could differentiate healthy, gingivitis and periodontitis. The detection accuracy of the chair-side test strip were on par with ELISA (sensitivity 92.9% and specificity of 100%) which were statistically significant ($P < 0.05$). A desire to arouse interest about periodontal health and maintenance in the Indian population provided a strong rationale for us to develop our chair-side test strips to suit our economy. Moreover, this was the first ever effort to develop and validate a chair-side test strip to detect MMP-8 levels in the Indian population. This test can be used on a large scale in private dental practice for the early detection of disease, tapping the sites at risk for disease, alongside helps in patient education and motivation for maintenance.

CONCLUSION: This study shows that the novel chair side test kit detects MMP-8 levels a biomarker of periodontal disease progression accurately making it a good chair side diagnostic tool. Further, it is cost effective and time saving which can make it applicable in private dental practice on a large scale for the early detection of periodontal disease.

Key words: Chair-side test; Chronic periodontitis; Gingival crevicular fluid; Matrix metalloproteinase-8; Periodontal health

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INTRODUCTION

Periodontal diseases are chronic inflammatory diseases of

the supporting structures of the teeth. Though periodontitis is often triggered by periodontopathogens, its clinical outcome is highly influenced by the host local immune response[1]. In view of the irreversible nature of progressive periodontitis, an early diagnosis and treatment of this disease is important. An early diagnosis will help prevent further irreversible loss of connective tissue attachment of teeth and adjacent alveolar bone associated with periodontal disease[2,3]. It is a well-established fact that the host immune products in periodontitis are synthesized locally and appear within the gingival crevicular fluid (GCF). This makes GCF ideal for obtaining diagnostic information of periodontal health or disease status[4]. The markers thus identified include cytokines, prostaglandins, bacterial- as well as host-derived enzymes, and connective tissue-degradation products, alongside bone matrix components that are primarily isolated in the GCF[5].

Matrix metalloproteinases (MMPs) are one such Group of enzymes which play a key role in the mediation of tissue destruction in periodontitis. MMP-8, a collagenase synthesized by neutrophils, is the major metalloproteinase implicated in the degradation and remodeling of the extracellular matrix. MMP-8 has a strong affinity towards type I collagen, which is present in abundance in the periodontal tissues[6]. There is growing evidence which indicate that a predominant association exists between increased GCF collagenase activity and disease progression, as it is extensively distributed in diseased periodontal tissues. A significant decrease in the GCF MMP-8 activity has been demonstrated following successful non-surgical periodontal therapy[7,8]. The potentiality of MMP-8 as a biomarker of periodontal disease progression is evident from literature.

Keeping this in view, the first chair-side, point-of-care dip-stick test based on the principle of immunochromatography utilizing monoclonal antibodies was developed and tested successfully to detect MMP 8 levels in GCF[9]. However, it was not popularized to be used on a large scale in private dental practice, probably because there were no further studies reported in literature evaluating and validating its use on a large sample size; Secondly, cost of the test kit could have escalated with the use of monoclonal antibody.

This fact provides a strong clinical rationale to indigenously develop a point-of-care test utilizing polyclonal antibody which is cost effective compared to monoclonal based test kit for MMP-8 detection with a diagnostic accuracy on par with enzyme-linked immunosorbent assay (ELISA). To overcome this limitation we indigenously developed a novel chair side point of care dip stick test based on the principle of immunochromatography utilizing polyclonal antibody (cost effective) instead of monoclonal antibody to detect MMP-8. The objective of the current study is to validate diagnostic accuracy (sensitivity and specificity) of our indigenously developed a novel chair-side test kit compared to that of ELISA in periodontal health and disease. Further to check the cost-effectiveness to suit the economies of developing countries like India.

MATERIALS AND METHODS

Study population

The study population consisted of 187 subjects (77 males and 90 females), 30-39 years of age, who were screened from the outpatient section of Department of Periodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore and 150 subjects (75 males and 75 females) were recruited for the study that was conducted during April to May 2010.

The following criteria prevented the patients from being included in the study Groups: medically compromised patients requiring prophylactic antibiotics, patients on antibiotic therapy within the last 6 mo, patients who had received any form of periodontal therapy surgical or non-surgical within 6 mo of baseline examination, smokers, pregnant patients, patients with recent orthodontic treatment, pulpal or periapical involvement on the qualifying teeth.

Each subject underwent a full mouth periodontal probing and charting, the subjects were categorized into three Groups based on clinical examination of gingival index (GI) (Loe and Silness, 1963), plaque index (PI) (Turesky Gilmore Glickman modification of the Quigley and Hein plaque index, 1970) and probing pocket depth (PPD) using a UNC15 probe. Fifty subjects with clinically healthy periodontium, mean PI $\leqslant$ 1, mean GI $\leqslant$ 1, no PPD, were included in Group 1. Group 2 (gingivitis Group) consisted of Fifty subjects with gingival inflammation as indicated by the mean PI $\geqslant$ 2, GI $\geqslant$ 2 and the absence of PPD. Group 3 (chronic periodontitis) consisted of fifty subjects with a mean PI $\geqslant$ 2, GI $\geqslant$ 2 and PPD $\geqslant$ 5 mm falling in the category of severe periodontitis as per the classification. Therefore of the patients recruited in the study, Group 1 had individuals with a healthy periodontium, Group 2 included individuals with gingivitis and no attachment loss and those in Group 3 were diagnosed to have severe chronic periodontitis. Groups 2 and 3 were treated with non surgical approach, scaling and root planning was done using area-specific Gracey currets (Hu friedy) and ultrasonic scalers.

Subjects satisfying the above criteria for enrolment were selected consecutively, and ethical clearance for the study was obtained from the institutional ethical review board, Rajiv Gandhi University of Health Sciences, in accordance with the guidelines of Indian council of Medical research. Written informed consent as per the declaration of Helnski 2008 was obtained from those who agreed to participate in the study.

Site selection and collection of GCF

The study was a triple blind prospective cross-sectional study in which clinical examination, Group allocation and sample site selection was performed by one examiner, the

samples were collected on the subsequent day by the second examiner, and a third examiner carried out the post-treatment clinical examination. This was done to ensure masking of the sampling examiner and to prevent contamination of GCF with blood associated with the probing of inflamed sites. One site per subject was sampled, in gingivitis patients, site with most severe clinical inflammatory signs (in gingivitis cases) or greatest amount of probing depth (in chronic periodontitis cases), along with radiographic confirmation of alveolar bone loss, and the same test site was selected for the after-treatment Group.

On the subsequent day, after drying the area with blast of air, supragingival plaque was removed without touching the marginal gingiva to eliminate the possibility of saliva contamination and thereafter GCF was collected. A standardized volume of 3 μL was collected from each test sites using the calibration of colour coded 1-5 μL calibrated volumetric microcapillary pipettes (Sigma Aldrich, St. Louis, MO) with an extracurricular (unstimulated) method. The test site, which did not express any volume of GCF, and microcapillary pipettes suspected of being contaminated with blood and saliva were excluded from the study. In such cases the sample was obtained from the tooth that showed the next highest PPD in the same patient. The GCF collected was transferred to eppendorf tubes containing 0.5 mL of phosphate buffer saline and stored at -70 ℃ until the time of assay.

Though cumbersome this method of GCF collection was adopted as to prevent protein binding to the paper strips and the risk of sample evaporation.

MMP-8 assay

The samples were assayed for MMP-8 levels using commercially available ELISA kit. The assays were conducted according to the manufacturer's instructions. Highly sensitive ELISA kit (Booster Biological technology Co., LTD, Shanghai) was used to detect the enzyme levels in the sample. This kit reported an assay of sensitivity of < 10 pg/mL. In relation to specificity, the manufacturer reported no significant cross reactivity or interference for the ELISA kit. The samples were run in duplicates. The kit made use of biotinylated anti-human MMP-8 antibody and Avidin-Biotin-Peroxidase Complex. Absorbance of the substrate colour reaction was read on ELISA reader using 450 nm wavelengths. The total MMP-8 level was determined in nanograms (ng), and the calculation of the concentration in each sample was performed by dividing the amount of enzyme by the volume of sample (ng/mL).

Fabrication of point-of-care test sticks for chair-side monitoring

The chair-side test was fabricated based on the sandwich ELISA principle. Nitrocellulose membrane of pore size 0.45 μm was cut into small strips. One end of the strip was treated with methanol to make the membrane hydrophilic. Methanol was washed off using 0.01 mol/L phosphate buffered saline (PBS) buffer. 1 μL of a primary polyclonal antibody to human MMP-8 was added to the hydrophilic end of the nitrocellulose membrane and allowed to dry. GCF sample collected was transferred to an eppendorf tube containing 0.5 mL of 0.01 mol/L PBS buffer. The test strip was immersed into this tube to allow the sample to bind the primary antibody. The strip was removed and washed thoroughly in the PBS buffer to remove the unbound sample. The strip was then dipped in an eppendorf tube containing secondary antibody to human MMP-8 conjugated with a peroxidase system for 10-15 min. The strips were washed in the buffer again and transferred to another eppendorf tube to which a colour developing solution was added. The solution turns blue in colour for samples positive for MMP-8. A colour change within 5 min was recorded as +++ (strongly positive), a change between 5 to 10 min was recorded as ++ (moderately positive) and a change in colour after 15 min was recorded as + (weakly positive). The individual reading the test results was unaware of the ELISA results to eliminate bias and ensure blinding.

Statistical analysis

Analysis of variance was used to compare all variables between groups and a P value of $\leqslant 0.05$ was considered to be statistically significant. SPSS version 13 was used for all the analysis.

RESULTS

Recruitment of subjects for the study started in the first week of April 2010, recruitment ended by the end of the second week of April. 187 subjects (77 males and 80 females), 30-39 years of age, were screened from the outpatient section of Department of Periodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore. Of the 187 individuals screened only one hundred and fifty subjects (75 males and 75 females) were available for analysis. GCF MMP-8 levels measured by enzyme linked immune sorbent assay were able to distinguish sites with periodontitis from those with gingivitis and healthy sites. All samples in each Group tested positive for MMP-8. The highest mean concentration of MMP-8 was obtained in Group 3 (1948.65 ± 916.44 mg/mL), and the lowest mean concentration was obtained in Group 1 (96.90 ± 30.88 mg/mL). The mean MMP-8 concentration for Group 2 (797.94 ± 185.60 mg/mL) was intermediate between the healthy and periodontitis sites. GCF MMP-8 levels > 1 mg/mL especially helped to differentiate the periodontitis from gingivitis and healthy sites, and 1 mg/mL was used as cut-off point in the chair-side test as previously reported (Tables 1 and 2).

A significant improvement in clinical parameters was observed after treatment (Table 3). κ statistics were performed to know the degree of agreement between the test stick and ELISA results. κ value was 0.959, indicating

Table 1 Descriptive data showing comparison of enzyme-linked immunosorbent assay and test stick results

Visit	Group	Test stick results	MMP-8 (mg/L)		Total
			> 1	≤ 1	
Baseline	Group 1	Positive		1	1
		Negative		49	49
		Total		50	50
	Group 2	Positive	4	0	4
		Negative	8	38	46
		Total	12	38	50
	Group 3	Positive	30	2	32
		Negative	5	13	18
		Total	35	15	50
3 mo	Group 1	Positive		1	1
		Negative		49	49
		Total		50	50
	Group 2	Negative		50	50
		Total		50	50
	Group 3	Positive	13	0	13
		Negative	0	37	37
		Total	13	37	50

MMP: Matrix metalloproteinase.

Table 2 Test strip results at baseline and three month follow-up

Visit	Group	Site	Test result of site	≤ 1 mg/L	> 1 mg/L
Baseline	Group 1	50	Positive	1	0
			Negative	49	0
	Group 2	50	Positive	0	4
			Negative	38	8
	Group 3	50	Positive	2	30
			Negative	13	5
3rd mo	Group 1	50	Positive	0	1
			Negative	0	49
	Group 2	50	Positive	0	0
			Negative	0	50
	Group 3	50	Positive	0	13
			Negative	37	0

a good agreement between the two tests at both baseline as well as the third month follow-up.

DISCUSSION

MMP-8 or collagenase-2 is one of the central biomarkers in the breakdown of periodontal connective tissue during the transition from health to disease[7,10-13] besides it has been found to be a potential candidate for use in diagnostic aids[14,15].

A triple blind prospective study was done to gain an insight into the diagnostic accuracy of the MMP-8 chair-side test and check its cost-effectiveness for application on a large scale. A possible role of MMP-8 as a mediator of periodontal inflammation and a comparison of the levels of MMP-8 in GCF among the three study Groups namely Group1, Group 2, and Group 3 and after treatment in Group 2, and Group 3 was assessed using our chair-side point of care test as well as ELISA. Since there

Table 3 Changes in clinical parameters from baseline to three months following scaling and root planning *n* (%)

Visit	Bleeding on Probing	Probing pocket depth		Total
		≥ 5 mm	< 5 mm	
Baseline	Negative	8 (30.8)	8 (33.3)	16 (32.0)
	Positive	18 (69.2)	16 (66.7)	34 (68.0)
	Total	26 (100.0)	24 (100.0)	50 (100.0)
3 mo	Negative		43 (86.0)	43 (86.0)
	Positive		7 (14.0)	7 (14.0)
	Total		50 (100.0)	50 (100.0)

have been reports on age-dependent changes in inflammatory mediators, we selected subjects in the age Group of 30-39 years to control the influence of age on the levels of MMP-8[16]. Further, a single site was selected for sample collection from each participant, which precluded the pooling of samples from multiple sites. Unstimulated samples were collected as an increase in vascular permeability of the blood vessels following gingival stimulation has been reported[17], suggesting that the levels of MMPs in GCF could be influenced by stimulation in sampling.

To the authors' knowledge, this study was the first of its kind to investigate the GCF levels of MMP-8 in the Indian population. Furthermore, this was the first attempt to tailor a chair-side diagnostic test to detect these enzyme levels in Indian population and suit their economic status as well. Though previous studies have successfully designed a chair-side test it had few shortcomings, firstly, the cost of the test strip which was escalated due to the use of monoclonal antibodies. Second, there was no data on post-treatment assessment of MMP-8 levels using the chair-side test which would have been of great help in patient education and motivation, and to identify the site at risk for disease progression.

The main finding of our study was that a strong association exists between the MMP-8 levels and the degree of inflammation as indicated by the changes in clinical parameters and the enzyme levels. The chair-side test strips also showed good agreement in this accord as depicted by the κ values. The specificity and sensitivity of the test strip were found to be good (sensitivity 92.9% and specificity of 100%).

Test strips were fabricated using polyclonal antibodies instead of the monoclonal antibodies previously used, this cut down the cost of the chair-side test almost threefold. Moreover, the specificity and sensitivity remained on par with the previously designed test, this further confirmed that the specificity and sensitivity obtained with either polyclonal or monoclonal antibodies remained almost the same[18] validating our results.

Even though ELISA is a highly sensitive assay and is widely used to detect various biomarkers to aid in diagnosis of various diseases there were certain limitations of ELISA, like technique sensitivity, the time consuming, a delay in providing results to the patients and the cost

involved favoured the fabrication of an easy to use chair-side test strip.

Our chair-side test strip confirmed that it serves as a good diagnostic tool and helps in early detection and maintenance of patients. However, due to the cross-sectional setting of the present study no definite conclusion can be drawn in this regard. To overcome this limitation of the present study, more number of multicenter trials need to be carried out with larger sample sizes. In the near future the chair-side tests could help in the diagnosis of at risk sites in periodontal patients as well as in the early detection and control of periodontal disease in patients at risk due to various systemic and environmental factors. Currently, a randomized controlled trial on a large sample size is being carried out by our organization to tap the at risk population with our new chair-side diagnostic test strip for MMP-8 detection. Apart from this another trial using the chair-side MMP-8 test is being used to detect MMP-8 levels in saliva samples as well to make it less cumbersome and more cost effective.

In short, it can be said that MMP-8 levels reflects the levels of inflammation in the tissues and this can be maintained at low levels with good patient education and regular maintenance. Currently, ELISA is widely used to detect biomarkers of various diseases. But it has some limitation like technique sensitivity and time consuming and cannot be adopted as a chair-side diagnostic aid. The quest to develop a rapid chair-side diagnostic aid with high validity prompted us to fabricate an indigenous chair-side point of care test.

COMMENTS

Background

Periodontitis is a complex, multifactorial disease, whose progression depends on the interplay between periodontopathogens, environmental factors and the host response. Matrix metalloproteinase-8 (MMP-8) is one of the major enzymes of host response to influence the degradation of the periodontal connective tissues. Many studies have proved this role of MMP-8, a chair-side test kit was also fabricated but was not popularized.

Research frontiers

The detection of MMP-8 by various methods has been carried out regularly. However, a point- of-care chair-side test with diagnostic accuracy on par with the regular methods like enzyme-linked immunosorbent assay as well as economic stability was lacking in developing countries like India. In this study, the authors demonstrated the accuracy of an indigenously prepared chair-side test which was suitable for large scale private practice use as well.

Innovations and breakthroughs

This study confirmed that a strong association exists between the MMP-8 levels and the degree of inflammation as indicated by the changes in clinical parameters and the enzyme levels. The chair-side test strips also showed good agreement in this accord as depicted by the κ values. The specificity and sensitivity of the test strip were found to be good (sensitivity 92.9% and specificity of 100%). Therefore it serves as a good diagnostic tool and helps in early detection and maintenance of patients.

Applications

Thus the indigenously fabricated test can be used along chair-side to detect the MMP-8 levels and identify at risk sites and patients thus aiding in an early diagnosis and good maintenance of periodontal patients.

Terminology

MMPs are matrix metalloproteinases, which represent a Group of Zinc dependent enzymes involved in connective tissue degradation. Of these MMP-8 is the major enzyme with an affinity towards collagens.

Peer review

This study is interesting and the results are useful to the readers. The authors concluded that the novel chair side test kit detects MMP-8 levels a biomarker of periodontal disease progression accurately making it a cost effective and time saving diagnostic tool. It really adds new information. It is a prospective well designed research study with interesting results. The paper is well written with appropriate structure. Thus, it could be accepted for publication.

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases.

Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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BRIEF ARTICLE

Quantitative scintigraphic analysis of the apical seal in Thermafil/Topseal and RealSeal 1/Realseal filled root canals

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Abstract

AIM: To investigate the microleakage of two different root canal obturation systems, using the nuclear medicine approach.

METHODS: Twenty-six single-rooted extracted teeth were selected. The crowns were sectioned to obtain 15-mm long root segments and each tooth was prepared using rotary ProFile® instruments. The roots were divided into 2 experimental groups using RealSeal 1 and RealSeal sealer or Thermafil and TopSeal sealer as well as two control groups. On the 7th and the 28th day the apices were submersed in a solution of ^{99m}Tc-Pertechnetate during 3 h. The radioactivity was counted using a γ camera.

RESULTS: The present study showed that none of the root canal-filled teeth was leakage free. The statistical analyses were made using Kruskal-Wallis and statistical significance was assessed using $\alpha = 0.05$. Although apical leakage measured in counts per minute (cpm) in the Thermafil/TopSeal group was lower than in the RealSeal/RealSeal group (363 916 ± 180 707.7 cpm *vs* 533 427 ± 414 020.6 cpm) on 7th day and (1 678 200 ± 567 217.4 cpm *vs* 2 240 518 ± 383 356.7 cpm) on 28th day, there was no statistical difference ($P > 0.05$). In the Thermafil/TopSeal group and RealSeal 1/RealSeal group it was found that over time, the number of counts increased between 7 d and 28 d (363 916 ± 180 707.7 cpm *vs* 1 678 200 ± 567 217.4 cpm) and (533 427 ± 414 020.6 cpm *vs* 2 240 518 ± 383 356.7 cpm), respectively, with statistically significant differences (Thermafil/TopSeal group, $P = 0.015$ and RealSeal 1/RealSeal group, $P = 0.036$).

CONCLUSION: Both carrier-based Realseal 1 and Thermafil techniques showed a similar sealing effect, but none of the materials was leakage free.

Key words: Microleakage; Nuclear medicine; Obturation; Thermafil; Realseal

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INTRODUCTION

It has been established that apical periodontitis is caused by bacteria derived from the root canal[1,2]. The first stage of root canal therapy is microbial control, followed by root canal filling. Microbial control includes removal of the protein degradation products, toxins, and mainly bacteria[3]. The root canal filling must seal the canal space both apically and coronally and the most commonly used material for root canal obturation is gutta-percha combined with a sealer. Gutta-percha is considered an imper-

meable core material but does not bond to root dentin walls.

Recent studies have shown that the obturation system with Resilon is able to create a monoblock which prevents bacterial leakages *in vitro* and *in vivo*[4,5]. In addition, it also increases the fracture resistance of the filled roots[4,6].

Methacrylate resin-based sealers are used in endodontics for improving bonding to radicular dentin. They are based on dentin adhesion techniques derived from restorative dentistry[7]. However, effective bonding within a deep and narrow canal is a challenge, mainly because of the unfavorable geometry of the root canal system to relief of polymerization shrinkage stresses[8,9].

Chemical irrigants are essential for successful debridement of root canals, during shaping and cleaning procedures which aim to expose the collagen networks. It has been shown that the removal of the organic phase from the mineralized dentine by NaOCl enhances dentin permeability to ethylenediaminetetraacetic acid (EDTA). Additionally, removal of the smear layer has been recommended in order to reduce microleakage and improve the fluid-tight seal of filled canals[10-12]. For this purpose, EDTA has been commonly used and is recommended by manufacturers as the final irrigant before the application of methacrylate resin-based sealers[13,14].

To date, several studies have evaluated the outcomes of different root canal sealers with various leakage models[15-18]. The major problem of most laboratory-based leakage testing models is that the obtained data are qualitative rather than quantitative, raising doubts about their reliability[15-17,19].

The use of sodium pertechnetate (^{99m}TcNaO$_4$) in nuclear medicine is well established and the evolution of diagnostic conventional nuclear medicine can be mainly attributed to the existence and the chemical versatility of this radionuclide[20,21].

The most relevant property of ^{99m}Tc is its 140 keV γ photon emission with 89% abundance, which is optimum for imaging with the γ cameras used in nuclear medicine. Moreover, its half-life of 6 h is enough to prepar-e the radiopharmaceuticals, to perform their quality control, and to administer them to the patient for imaging studies, while having a favorable dosimetry. The rapid growth in this field in the last few decades is attributable, in addition to its ideal radionuclide characteristics, to the design and development of ^{99}Mo/^{99m}Tc generators and lyophilized kits to facilitate the formulation of ^{99m}Tc compounds in hospital radiopharmacies. ^{99m}TcNaO$_4$ is obtained in the form of sodium pertechnetate directly from the generator after elution with saline. Given all the ^{99m}Tc characteristics and considering that nuclear medicine is an approach with high sensitivity and specificity, radionuclides may provide quantitative and objective results concerning infiltration.

Thus, the aim of this study was to use nuclear medicine methodologies to assess microleakage of root canals. For that purpose we compared the sealing ability of roots filled with Realseal 1/Realseal, with those that were filled with gutta-percha and an epoxy-based root canal sealer (Thermafil/Topseal) using the ^{99m}TcNaO$_4$.

MATERIALS AND METHODS

Twenty-six extracted human premolar teeth, with a single root and the apex completely formed were used in this study. These teeth were stored until use in a 0.9% sodium chloride solution containing 0.02% sodium azide at 4 ℃, to prevent bacterial growth.

The crowns were sectioned with a high-speed bur and water spray, in order for all the roots to be approximately 15 mm long. Canal length was determined by inserting a K file, ISO size #15 (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) into the canal until its tip was visible at the apical foramen. The working length was established 1 mm short of the apex.

Instrumentation of the root canals was performed with a crown-down technique using ProFile nickel-titanium rotary instruments (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland). The handpiece was used with an electric motor (X-Smart; Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) at 250 rpm. Instrumentation was completed with 35.04 ProFile instruments up to the working length. After the use of each instrument, the canals were irrigated with 3 mL of 2.5% NaOCl by using a 27-gauge Monoject irrigation needle (Sherwood Medical, St. Louis, MO). The final rinse was performed using 3 mL of 17% EDTA for 3 min, and 3 mL of 2.5% NaOCl for 3 min (Pulpdent Corporation, Watertown, MA) followed by 3 mL of saline solution for 1 min. The canals were dried with size 35 absorbent paper points (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland). Finally, the roots were randomly divided into 2 experimental groups of 10 teeth each (group 1 and 2) and two control groups of 6 teeth. In group 1 the obturations were performed with TopSeal sealer (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) placed into the canal using a 35.04 master points. Then, the Thermafil (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) carrier points 35.04, was inserted in the canal after being thermo plasticized in the ThermaPrep oven (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland), according to the manufacturer's instructions. The excess of gutta-percha was removed with a hot instrument, and then the root canal orifices were sealed using a flowable resin composite (Vertise Flow, Kerr SA, 6934 Bioggio, Switzerland).

As for group 2, the obturations were performed by using 35.04 RealSeal 1 carrier points. The RealSeal primer was placed into the root canal with a microbrush, and the excess of primer was removed with paper points. Then, the RealSeal SE sealer was placed into the canal with a #35 K-file. The RealSeal 1, 35.04, was inserted in the canal after being thermo plasticized in the Realseal 1 oven (SybronEndo). After canal filling, the coronal surface of the root filling was light cured for 40 s (Optilux; Sybron Kerr, Danbury, CT), the excess of obturation material was removed with a round bur and the root canal orifices were sealed using a flowable resin composite (Vertise Flow, Kerr SA, 6934 Bioggio, Switzerland).

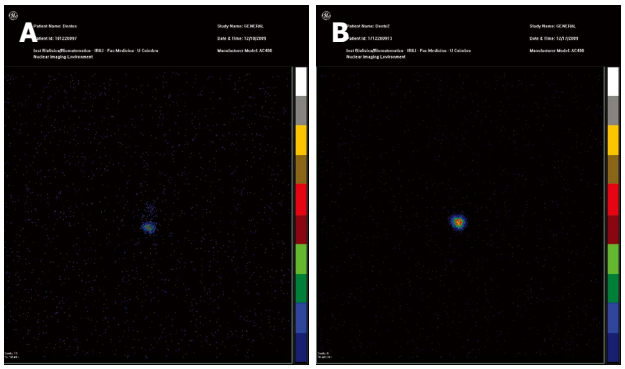

Figure 1 In each image regions of interest were drawn over each tooth, to obtain the counts/min. A: Nuclear Medicine static image obtained 3 h after $^{99m}TcNaO_4$ infiltration of filled roots with Thermafil/TopSeal; B: Nuclear Medicine static image obtained 3 h after $^{99m}TcNaO_4$ infiltration of filled roots with RealSeal 1/RealSeal.

All experimental procedures were carried out by the same endodontist.

After the filling procedures, two radiographs were taken in orthoradial and proximal views, to analyse the quality of the canal filling.

The filled root segments were stored for 1 wk at 37 ℃ and 100% relative humidity to allow the sealers to set completely, before leakage evaluation using a nuclear medicine approach.

For the control group (n = 6), procedures for selection and instrumentation were the same as those described for the experimental groups, except that the prepared root canal space was not obturated in the positive control group.

Evaluation of leakage

For leakage determination, the roots of the experimental groups and positive control group were covered by two layers of nail varnish, except for 2 mm of the apical foramen. In the negative control group, the entire root surface, including the apical foramen was covered by the nail varnish. The teeth were suspended in Eppendorf tubes, containing $^{99m}TcNaO_4$ remaining in contact with the solution for 3 h. After this period the roots were dried on absorbent paper and the varnish was then removed. Scintigraphic images were made for each tooth using a γ camera (GE 400 AC, Milwaukee, United States). For each tooth a static image was acquired for 3 min at a 512 × 512 matrix size (Figure 1). Regions of interest (ROIs) in each image were drawn over each tooth, to obtain the total counts and the counts per minute (cpm). All the nuclear medicine procedures were performed by a single nuclear medicine specialist, in a blinded fashion. The procedure was repeated at 7 and 28 d.

Statistical analysis

The statistical analyses were cariied out using the Kruskal-Wallis method and statistical significance was assessed using $\alpha = 0.05$, using statistics software (SPSS version 19, Japan Inc., Tokyo, Japan).

Table 1 Mean values and SD of counts per minute and scores for each root for each time

Day	Group	*n*	Mean (cpm)	StDev (cpm)
D7	Ctr +	3	1 072 039	561 126.9
	Ctr -	3	95 881.0	117 789.4
	Group 1[a]	10	363 916	180 707.7
	Group 2[b]	10	533 427	414 020.6
D28	Ctr +	3	2 328 358	112 295.3
	Ctr -	3	352 275.7	340 167.6
	Group 1[a]	10	1 678 200	567 217.4
	Group 2[b]	10	2 240 518	383 356.7

Group 1: Thermafil/TopSeal; Group 2: RealSeal 1/RealSeal; Ctr +: Positive control; Ctr - : Negative control. [a]P = 0.015, [b]P = 0.036.

RESULTS

The means and standard deviations of ROI cpm from the specimens at 7 and 28 d after obturation are given in Table 1. In group 1 (Thermafil/TopSeal, Figure 1A), it was found that over time, the number of counts increased between 7 and 28 d: 363 916 and 1 678 200 cpm, respectively. These differences are statistically significant (P = 0.015, Table 1). In group 2 (RealSeal1/RealSeal, Figure 1B) statistically significant differences were found between the counts obtained at 7 and 28 d: 533 427 and 2 240 518 cpm, respectively (P = 0.036, Table 1). Comparing the core/sealers, it was found that although the Thermafil system had less leakage than RealSeal 1, there was no statistically significant difference.

DISCUSSION

Analysis of the sealing ability of new root canal obturation systems is important, in relation to both the coronal and apical leakage, because they have been cited as a significant cause of post treatment disease[10,11].

Although a bacterial leakage model may appear to be more clinically relevant than a fluid infiltration model, the latter technique was used here[22].

Dye penetration is one of the most commonly used methods for the investigation of apical leakage because of the simplicity of both the laboratory procedures and the final reading of the results.

Although the fluid transport model/bacterial penetration has been widely used to determine leakage around coronal restorations and endodontic retrograde fillings[23] and has been proven to be more sensitive than conventional dye penetration, radionuclide methods are not usually used.

The principal advantage of using a radioactive probe with $^{99m}TcNaO_4$ is that this radionuclide method is quantitative and nondestructive, enabling measurement of microleakage from the same specimens at intervals over extended periods, without destroyed the sample. This procedure is important for the study of interfaces. With other methods, artifacts may occur at the surface level due to the section cutting process, but this does not oc-

cur with radionuclides and it is possible to evaluate leakage over extended periods.

Thermafil is a simple obturation method with short execution time, and which, according to the manufacturers, confers a good seal. According to Inan *et al*[24], obturation with systems carriers has a smaller variation in the values of leakage than vertical and lateral condensation, perhaps a good indicator for the best method for use in the clinic.

Because an properly filled canal requires an appropriate cleaning and shaping procedure, a 35.04 rotary file was used at 1 mm from the foramen. Better clinical antimicrobial efficacy using this diameter has been reported in the mandibular molar, than with a 30.04 diameter file when sodium hypochlorite was used[25].

Self-etch sealers, such as MetaSeal (Parkell, Farmington, NY) and RealSeal SE, have been incorporated recently into endodontic practice. This kind of sealer has been designed with the intention of combining a self-etching primer and a moderately filled flowable composite into a single product, to make possible adhesion to dentin substrates. These sealers are similar to self-adhesive luting cements, and it is assumed that the bonding mechanism is similar[26].

Furthermore, future studies using microscopic techniques should be performed to show if the Resilon or gutta-percha interfaces are able to avoid bacterial penetration, because few studies currently exist that verify *in situ* the presence of bacteria in samples showing leakage.

The findings of this study demonstrated that on the seventh day, specimens filled with RealSeal 1/RealSeal leaked more than specimens filled using Thermafil and TopSeal.

Similarly, RealSeal 1/RealSeal showed increased leakage at all times, irrespective of the obturation technique with Thermafil/TopSeal. Although adhesive products hold promise for the future, a number of technical hurdles need to be overcome in order to maximize the potential benefits of adhesive root canal fillings. One challenging concern is the very limited capacity of long narrow root canals to relieve polymerization shrinkage stresses created by methacrylate-based sealers *via* resin flow[27]. This may be expressed in terms of the cavity configuration factor or C-factor, being the ratio of the bonded surface area in a cavity to the unbonded surface area[28].

Under the experimental conditions of the current *ex vivo* experiment, the results demonstrated that the newly developed RealSeal 1/RealSeal core material combination of root canal filling materials does not improve the microleakage resistance compared with Thermafil/Topseal filling. Nevertheless, further investigation of other features of root canal sealers is required.

COMMENTS

Background

The sealing ability of root canal obturation systems is important, in both the coronal and apical leakage. Gutta-percha is considered an impermeable core material but does not bond to root dentin walls. Recently, a resin-based obturation system, Real Seal was introduced as an alternative to gutta-percha. It consists of a resin material, named Resilon. Many studies have reported that the obturation system with Resilon and Methacrylate resin-based sealers is able to prevents bacterial leakages *in vitro* and, in addition, increases the fracture resistance of the filled roots.

Research frontiers

Thermafil is a simple obturation method with short execution time, and which, according to the manufacturers, confers a good seal. Self-etch sealers, such as MetaSeal (Parkell, Farmington, NY) and RealSeal SE, have been incorporated recently into endodontic practice. This kind of sealer has been designed with the intention of combining a self-etching primer and a moderately filled flowable composite into a single product, to make possible adhesion to dentin substrates, thereby preventing microleakage.

Innovations and breakthroughs

Obturation with systems carriers has a smaller variation in the level leakage than with vertical and lateral condensation, perhaps a good indicator of the best method for use in the clinic. Previous findings have evaluated the outcomes of different root canal sealers with various leakage models. The major problem of most laboratory-based leakage testing models is that the obtained data are qualitative rather than quantitative, raising doubts about their reliability. The principal advantages of using a radioactive probe with $^{99m}TcNaO_4$ is that this method is a quantitative and nondestructive method, enabling measurement of microleakage from the same specimens at intervals over extended periods, without destroying the sample.

Applications

The study results suggest that the RealSeal 1/RealSeal core material combination could potentially be used for root canal filling.

Peer review

This is an interesting study, and is well written for publication.

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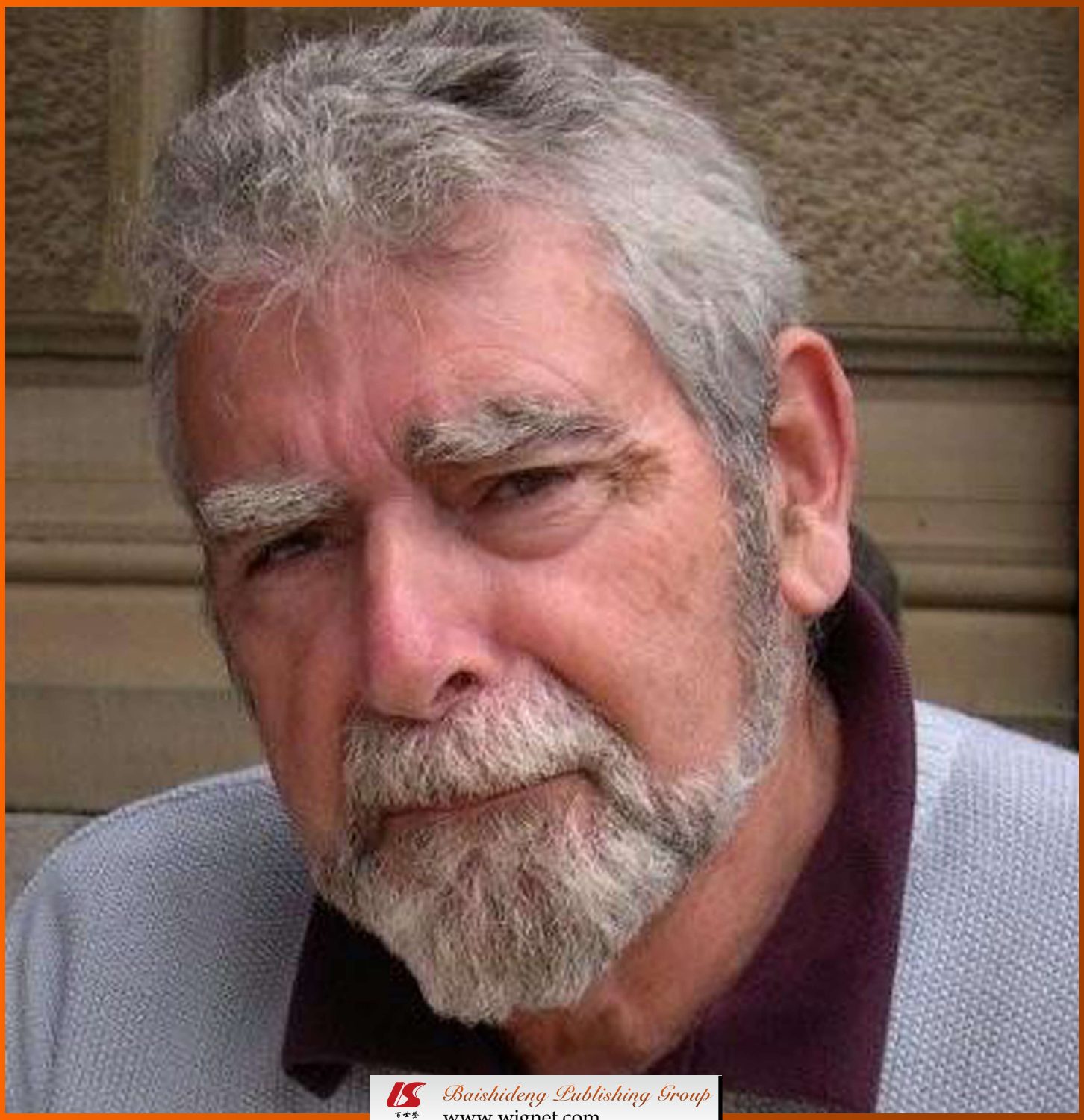

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MINIREVIEWS

Dental stem cells: Progress and perspectives

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Abstract

Dental pulp stem cells (DPSCs) are thought to contribute to reparative dentin formation, and that they may correspond to heterogenous populations of precursor cells or represent distinct differentiation stages along the odontoblastic lineage. DPSCs share many similarities with mesenchymal stem cells of the bone marrow (BMSCs). It appears that the distribution of tissue stem cells is not random and, within the dental pulp, there are potentially several distinct niches of stem/progenitor cells. In addition to DPSCs, other dental stem cell populations have been isolated. As for DPSCs, further studies are still needed to evaluate their potential of differentiation and their regenerative activity. Up today, (1) the formal demonstration that pulpal resident stem cells are actually the reparative dentin-forming cells recruited in response to injury is still lacking; and (2) the origin, localization and precise identity of odontogenic stem cells remain largely unknown. Dental clonal cell lines may represent valuable tool to answer some fontamental questions concerning the dental stem cell biology. Altogether, the presence of dental cell populations displaying stem cell properties has opened new paths for considering regenerative therapies. This might be a prerequisite to design alternative strategies for capping and endodontic treatment, using stem cells.

Key words: Dental pulp; Stem cells; Dentin repair; Niche

Core tip: The presence of cell populations displaying stem cell properties within the pulp has opened new paths for considering more conservative therapies. Still, dental stem cells research is still confronted with the lack of precise knowledge related to the location and identity of the cells participating to reparative dentin formation. Clonal cell lines derived from the dental sphere may represent valuable tool to answer some questions that are of fundamental importance to stem cell biology and clinical applications. This review discusses some fundamental concepts of dental stem cell biology within the context of regenerative dentistry.

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INTRODUCTION

Tooth development requires a series of sequential and reciprocal interactions between the ectodermally derived oral epithelium at the origin of ameloblasts and neural crest-derived ectomesenchyme at the origin of odontoblasts. Tooth patterning proceeds through sequential morphogenetic events (bud, cap, bell) leading to crown and subsequently to root formation. During embryogenesis, morphogenesis is coupled to differentiation of commited cells that progressively elaborate enamel and dentin and in turn reach the terminal stages of amelogenic and odontogenic lignages. This cascade of events relies on

epithelial-mesenchymal interactions that progressely lead to transformation of the tooth germ into complex mineralized structures[1].

Ameloblasts are lost following enamel maturation and tooth eruption, and hence enamel cannot be regenerated. Dental papilla ectomesenchymal cells give rise to the embryonic pulp and odontoblasts. Dental pulp cells maintain tooth homeostasis and odontoblasts synthesize dentin extracellular matrix. Dentin and pulp are related embryologically, histologically, and are functionally associated although the term of dentin-pulp complex is a notion underlying an oversimplification.

Odontoblasts are polarized postmitotic cells. These terminally differentiated cells cannot undergo further cell division and proliferate to replace irreversibly injured odontoblasts. Only the postmitotic cells forming the sub-odontoblastic Hoehl's cell layer, have the capacity to acquire a polarized phenotype and become functionnal odontoblasts. Odontoblasts are responsible for the secretion of primary and secondary dentin. They have a natural regenerative potential leading to the formation of reactionary dentin[2]. Odontoblasts can be up-regulated to secrete a reactionary dentin matrix when a mild injury occurs, such as attrition or early caries. However, injury of greater intensity, such as deep caries or restorative procedures, may lead to the death of the pre-existing odontoblasts and Hoehl's cells[3]. In such cases, recruitment of stem/precursor cells within the pulp will give rise to a new generation of odontoblast-like cells capable to elaborate reparative dentin.

The process of dental tissue repair may share many similarities with the embryological mechanisms of tooth development. It is assumed that many genes and signaling pathways involved in odontogenesis are also implicated in the tooth repair. Still, the mechanisms underlying reparative dentin formation are "open research areas" and offer exciting opportunities for possible tooth regeneration and dental tissue engineering.

DENTAL PULP STEM CELLS

The post-natal dental pulp contains heterogeneous cell populations responsible for its maintenance, defence and capacity of repair: stromal fibroblasts, odonto-osteoprogenitors, neuronal and vascular cells as well as inflammatory and immune system cells such as dentritic cells, neutrophils, macrophages, lymphocytes[4]. The ability of the pulp to respond to a variety of pathological conditions and injuries by deposition of a reparative dentin by pulp "progenitors" is well recognized[5] but the origin, localization and precise identity of odontogenic stem cells remain largely unknown. Identifying cells mobilized in response to pulp injury is a prerequisite to design alternative strategies for capping and endodontic treatment, using stem cells.

A decade ago, a population of odontogenic progenitors, inferred as dental pulp stem cells (DPSCs), was isolated from the pulp of human permanent third molars[6]. Upon subcutaneous transplantation into immunocompromised mice, *in vitro* expanded DPSCs mixed with hydroxyapatite formed dentin/pulp like complexes at an ectopic site. Populations of DPSCs possesses (1) generic mesenchymal stem cells-like properties (MSCs); (2) colony forming ability; and (3) were shown to express *in vitro* osteoblastic, adipogenic, chondrogenic or even neuronal markers[7-9]. DPSCs share many similarities with mesenchymal stem cells of the bone marrow (BMSCs) which are the most studied stromal stem cell populations. More than 4000 human genes are expressed either by BMSCs or DPSCs[10]. Dental stem cell populations also express different panels of stem cell surface markers such as 3G5, STRO-1, CD44, CD106, CD146, CD90 and Sca-1 used to characterize hematopoetic stem cells. However, it is important to note that DPSCs and BMPCs have not the same embryonic origin and that cells derived from the human or animal dental pulps have not been able to support hematopoiesis in transplantation assays[11]. DPSCs are thought to contribute to reparative dentin formation, and it appears that they may correspond to heterogenous populations of precursor cells or represent distinct differentiation stages along the odontoblastic lineage.

The presence of cell populations displaying stem cell properties within the pulp has opened new paths for considering more conservative therapies[6]. Nevertheless, the formal demonstration that pulpal resident stem cells are actually the reparative dentin-forming cells recruited in response to injury is still lacking. The hypothesis that a subset of stem cells carried by the vasculature replenishes the pulp after lesion can not be totally excluded. In the pulp, as in most tissues, the size of the pool of stem cells is very small (< 1%) and little is known about their anatomical sites within the pulp[12]. Moreover, the responsiveness of the pulp provides a dynamic system for tissue repair that may imply migration of stem cells from their resting places to the site of injury. Undifferentiated mesenchymal/mesectodermal cells present in the stroma, perivascular cells such as Rouget's pericytes or fibroblasts have all been proposed as potential progenitors mediating pulp repair after destruction of the odontoblasts and the *Hoehl's* sub-*odontoblastic* cell *layer*[13]. Advances in imaging technology and identification of stem cell markers are still needed to visualize stem/precursor cells *in situ*.

WHERE ARE THE DENTAL PULP STEM CELLS NICHES?

Stem cells are rare cells that are uniquely capable of both reproducing themselves and generating the differentiated cell types that are needed to carry out specialized functions. Stem cell behaviour is regulated by inputs from their local environment often referred as the "stem cell niche". Niches are defined as specific anatomic locations that provide structural support, trophic support, topographical informations and the appropriate physiological cues to control the maintenance, quiescence, self-renewal, recruitment towards differentiation and long-term regenerative capacity of stem cells. Hallmarks of a niche may

include: the stem cell itself, stomal supporting cells that interact directly with the stem cells *via* secreted factors and cell surface molecules, extracellular matrix (ECM) that provides structure and mechanical signals, neuronal inputs and vascular network that carry systemic signals and represent a conduit for recruitment of inflammatory and circulating cells into the niche. In teeth, as in the adult blood system, multiple niches may exist and specific markers allowing the definitive identification of stem cells within the pulp are lacking.

Some data suggest that pericytes could differentiate into osteoblast-like cells, so odontogenic stem cells may reside in a perivascular niche[14]. In this context, it is interesting to mention that many haematopoetic stem cells (HSCs) and neuronal stem cells (NSCs) are localized close to the vascular network; this could be important to communicate "insult" and expose stem cells to signals activating their recruitment. Besides, alterations in ECM components and matrix elasticity related to damage or ageing may also provide mechanical signals that could have a profound impact on stem cell activity[15]. Thus, it appears that the distribution of tissue stem cells is not random and, within the dental pulp, there are potentially several distinct niches of stem/progenitor cells. Nevertheless, still little information is available regarding their topological organization and the inputs that recruit osteo-odontogenic stem cells to form reparative dentin[3]. In contrast to other tissues known to have a constant regeneration potential, such as intestine and bone marrow, dental pulp stem cells will react to form reparative dentin only after injury. This imply that signals ensure their survival and prevent their differentiation while maintaining their responsiveness following pulp damage. Whether an endogenous pool of stem cells associated with supportive stromal cells are mobilized at the site of injury and/or whether attraction of migrating stem cell is necessary to repopulate a niche and expand precursor cells at the appropriate site for dentin repair is unknown. In addition, the alteration of the dentin mineralized matrix promotes the release of bio-active molecules including high concentrations of Ca^{2+} which locally may also contribute to stem cells proliferation and differentiation in the post-injury pulpal environment.

DENTAL STEM CELLS-DIFFERENT TYPES, DIFFERENT LOCATIONS

In addition to DPSCs which were derived from the pulp of human permanent third molar, other stem cell populations have been isolated from exfoliated deciduous teeth (SHED), periodontal ligament (PDLCs), apical papilla (SCAP) and dental follicule (DFSCs). As for DPSCs, further studies are still needed to evaluate their potential of differentiation and their regenerative activity .

SHED, isolated from the pulp of human deciduous teeth appear distinct from DPSCs, having a higher proliferative rate and distinct gene expression profiles. SHED have osteoinductive capacity *in vivo*[16]. They can survive in mouse brain, expressing neural markers and possible application of SHED was even considered in alleviating Parkinson's disease[17]. As odontoblasts, they have a neural crest origin which may sustain their ability to adapt in a neuronal environment. Since children lose 20 deciduous teeth, SHED may be potentially used as an autologous stem cell source for dental pulp engineering once the children become adult. The commercial banking of these cells is becoming widespread but whether SHED maintain their stem cell properties after long-term storage (cryopreservation for more than 10 years) have not been assessed.

PDLSCs which derived from human periodontal ligament (PDL), a connective tissue between the cementum and the inner wall of the alveolar bone socket, represent a population of stem cells capable to differentiate in cementoblast-like cells and type 1 collagen-forming cells. Interestingly, transplantation of human PDLSCs in the periodontal defect of immunocompromised mice, promotes the formation of a periodontal -like tissue, suggesting that PDLCs may be a potential tool for alveolar bone repair[18].

SCAP are derived from the apical part of the papilla of growing tooth roots[19,20]. It is important to note that the apical papilla tissue is present while the root apex is still open, before tooth eruption. *In vitro*, SCAP have been shown to exhibit dentinogenic and adipogenic properties, they also express neuronal markers. In clinical practice, they are easily accessible from extracted wisdom molars which develop later in life. Whether SCAP may represent a source of autologous stem cells for tooth repair remains an open question.

DFSCs derived from the dental follicle, a fibrous ectomesenchymal tissue that surrounds the developping tooth germ during the crown formation and disappears during the early stages of root development[21,22]. This tissue will form the periodontium, *i.e.*, cementum, periondontal ligament and alveolar bone. Thus, DFSCs may correspond to a heterogenous cell populations with different type of stem cells including cementoblasts, osteoblasts, stromal cells. In the adults, DFSCs can be easily accessible in impacted wisdom tooth during crown formation but not later (Table 1).

Finally, since 2009, several publications describe new populations of mesenchymal stem cells isolated from the human oral mucosa and gingiva (Zhang *et al*, 2012). Their differentiation and therapeutic potentials remain to be determined.

PERSPECTIVES AND OPEN QUESTIONS

Dental stem cells research is still confronted with the lack of precise knowledge related to the location and identity of the cells participating to reparative dentin formation. To this end, our laboratory developed the strategy and established stem cell lines from embryonic pulp of transgenic mouse[23] and Figure 1. One of the clones has the capacity after implantation in a rat molar, and in the ab-

Table 1 Types of dental pulp stem cells and their properties

Type of stem cells after birth	Dental stem cell properties	Signaling inputs for reparative dentin formation
Stem cells permanently present in the adult tooth: Dental pulp stem cell Periodontal ligament stem cells Apical papilla stem cells Stem cells present in deciduous tooth: Exfoliated deciduous teeth stem cells Stem cells present during crown formation: Dental follicule stem cells	Self-renewal Ability to enter in mitosis in response to appropriate signals and to differentiate towards odonto/osteogenic cells Long-term survival and maintenance of reparative capacity Distinct subpopulations expressing markers of mesenchymal stem cells of the bone marrow (STRO-1, CD44, CD 106, 3G5, CD146, CD90, Sca-1…)	Tooth injury may promotes stem cell recruitment by: Local secreted factors: bioactive extracellular matrix molecules Ca^{2+} release Mechanical inputs: changes in matrix elasticity Diffusible signals emanating from stromal, inflammatory, circulating… cells

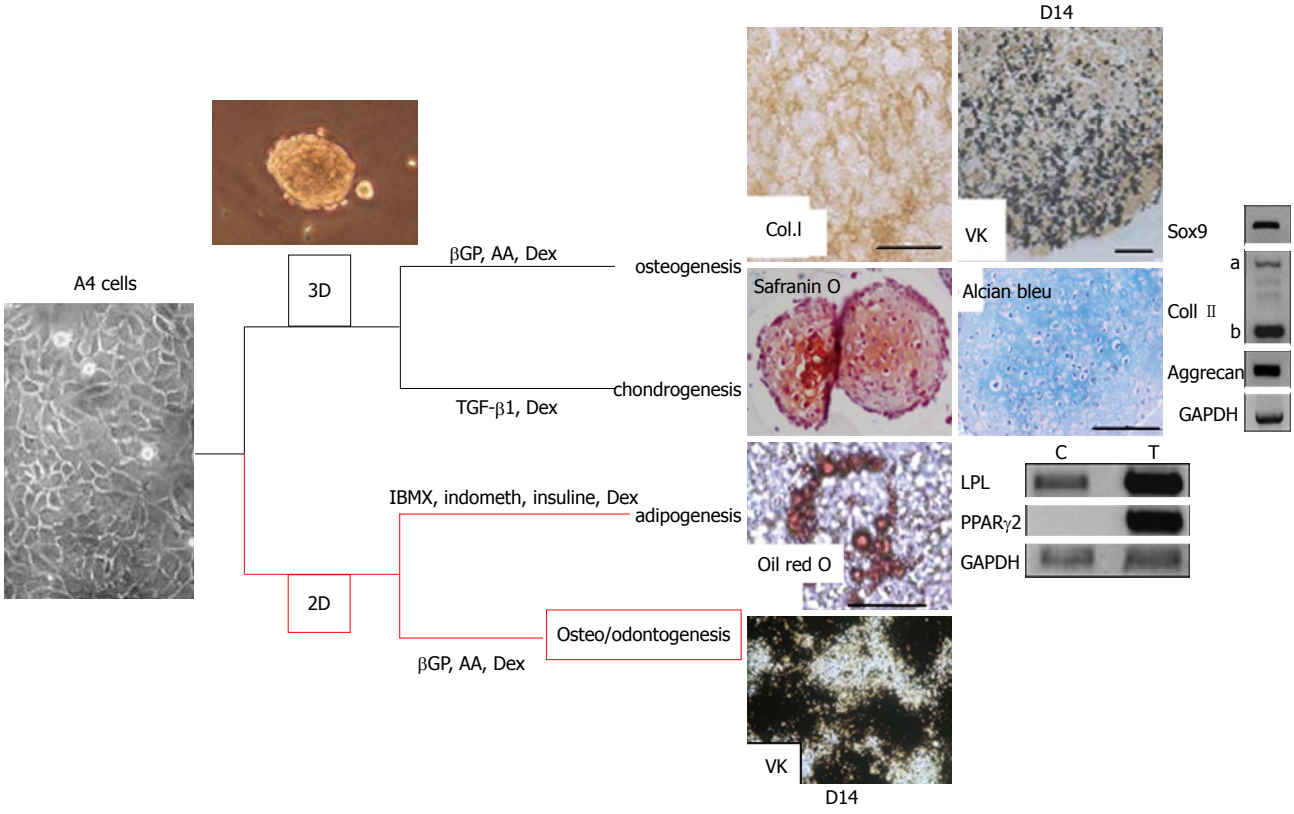

Figure 1 The A4 cells cultured in 2D or 3D, differently supplemented, give rise to different cell phenotypes, and consequently promote osteogenesis, chondrogenesis, adipogenesis or dentinogenesis. GAPDH: Glyceraldehyde phosphate dehydrogenase; TGF-β1: Transforming growth factor β1.

sence of any carrier or biomolecule, to promote efficient dentin repair[24,25]. Clonal cell lines derived from the dental sphere may represent valuable tool to answer several questions that are of fundamental importance to stem cell biology and clinical applications: Where are localized the presomptive stem cells niches? What are the markers allowing to visualize resident or migrating stem cells *in situ*? Which signals and molecular pathways sustain stem cells recruitment within the pulp and parodontium upon injury? By combining *in vitro* and *in vivo* experimental approaches, the answers to these questions will lead to a better understanding of stem cells potential for tooth repair and pave the way to develop new stem cell-based therapies.

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REVIEW

Basic properties and types of zirconia: An overview

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Abstract

This paper describes types and characteristics of zirconia materials in relation to their applications in dentistry. The zirconia material typically used today by most manufacturers is a tetragonal polycrystalline zirconia, partially stabilized with yttrium oxide. The mechanical properties of zirconia have been extensively investigated in the scientific literature and zirconia clearly measures up to any other equivalent manufactured material. The biocompatibility of zirconia has also been extensively evaluated and no local or systemic adverse reactions or cytotoxic effects have been found in relation to it. However, ceramic bonding, ageing, light transmission and manufacturing processes are all factors that need to be further evaluated in order to guide the successful use of zirconia as a prosthetic restorative material. Milling zirconia to full-contour might be an alternative to traditionally veneered restorations. A potential adhesion mechanism appears to be the combination of air abrasion with aluminum oxide particles (silanated or not), followed by sintering with materials containing special reactive monomers. Changes in zirconia properties before and after the sintering process have also been investigated. It was found that after sintering, surface roughness was greater, and micro hardness was slightly reduced; however, accurate precision of fit was not affected by the sintering process. Currently, zirconia restorations are manufactured by either soft or hard-milling processes, with the manufacturer of each claiming advantages over the other. Chipping of the veneering porcelain is reported as a common problem and has been labeled as its main clinical setback. As zirconia has demonstrated good mechanical and biological performance, future technology is attempting to improve esthetics and minimize veneer fracture, aiming to create confidence in the dental community towards this all-ceramic system. Milling zirconia to full-contour might be an alternative to traditionally veneered restorations. Finally, implications are drawn for manufacturing, machining, and widespread use of these materials.

Key words: Zirconia; Biocompatibility; Porcelain chipping; Mechanical properties

Core tip: Although all zirconia is chemically similar, the ultimate product can vary from manufacturer to manufacturer, with materials of varying density, uniformity homogeneity and crystalline transformation. This can be due to varying grain sizes of the powdered material ultimately affecting strength, with variations producing porosity. One type of restoration will not fit every clinical condition but today we have a range of options in zirconia ceramics, including monolithic full-contour type and conventional veneered type zirconia.

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INTRODUCTION

Zirconium (Zr) is a metal with the atomic number 40. It was first discovered in 1789 by the chemist Martin

Klaproth[1-3]. The material has a density of 6.49 g/cm³, a melting point of 1852 ℃ and a boiling point of 3580 ℃. It has a hexagonal crystal structure and is grayish in color. Zr does not occur in nature in a pure state. It can be found in conjunction with silicate oxide with the mineral name Zircon ($ZrO_2 \times SiO_2$) or as a free oxide (ZrO_2) with the mineral name Baddeleyite[4]. These minerals cannot be used as primary materials in dentistry because of impurities of various metal elements that affect color and because of natural radionuclides like urania and thoria, which make them radioactive[5]. Complex and time-consuming processes that result in an effective separation of these elements are necessary in order to produce pure zirconia powders. After purification the material produced can be used as a ceramic biomaterial[4,6,7].

ZrO_2 is a polymorphic material and occurs in three forms: monoclinic, tetragonal and cubic. The monoclinic phase is stable at room temperatures up to 1170 ℃, the tetragonal at temperatures of 1170-2370 ℃ and the cubic at over 2370 ℃[8,9]. However, noticeable changes in volume are associated with these transformations: during the monoclinic to tetragonal transformation a 5% decrease in volume occurs when zirconium oxide is heated; conversely, a 3%-4% increase in volume is observed during the cooling process[4,10] (Figure 1).

STABILIZED ZIRCONIA

Several different oxides are added to zirconia to stabilize the tetragonal and/or cubic phases. Magnesia (MgO), Yttria (Y_2O_3), Calcia (CaO), and Ceria (CeO), amongst others, allow the generation of multiphase materials known as Partially Stabilized Zirconia (PSZ), whose microstructure at room temperature generally consists of cubic zirconia as the major phase, with monoclinic and tetragonal zirconia precipitates as the minor phase[4,11,12].

PSZ materials have been tested for their potential use as ceramic biomaterials. Mg-PSZ is one of the most commonly used zirconia-based engineering ceramics[13]. Residual porosity in the mass of the material, a rather coarse grain size (30-40 μm), and difficulties in obtaining Mg-PSZ precursors free of impurities, are all factors that have discouraged the interest of ceramic manufacturers in the development of Mg-PSZ for biomedical applications[4]. It has been reported that reinforcement by phase transformation toughening is less pronounced in Mg-PSZ than in Y-TZP (Yttrium-Tetragonal Zirconia Polycrystals), a finding that is discussed below[13]. Ceria (Ce)-doped zirconia ceramics have been very rarely considered, although they exhibit superior toughness (up to 20 MPa) and show no signs of ageing[14].

TRANSFORMATION/TOUGHENING MECHANISM

In the presence of a small amount of stabilizing oxides, and at room temperature, it is possible to obtain PSZ ceramics in the tetragonal phase only, known as Tetragonal Zirconia Polycrystals (TZP). The finely dispersed tetragonal ZrO_2 grains within the cubic matrix, provided that they are small enough, can be maintained in a metastable state that is able to transform into the monoclinic phase[11]. Tetragonal-to-monoclinic phase transformation in zirconia can be induced by stress, temperature and surface treatments[15,16]. Low temperature ageing *via* phase transformation of zirconia hip joint heads in normal atmospheric conditions has been reported after 10 years of incubation[10]. After the ageing of yttrium-stabilized zirconium dioxide in body fluid or water, some tetragonal-to-monoclinic phase transformation on the surface of zirconium dioxide has also been reported[17,18]. Even though some phase transition does occur, reports indicate that the effect on the material's mechanical properties is negligible[4,10].

Y-TZP (YTTRIUM-TETRAGONAL ZIRCONIA POLYCRYSTAL)

The addition of approximately 2%-3% of mol yttria (Y_2O_3) as a stabilizing agent in zirconia allows the sintering of fully tetragonal fine-grained zirconia ceramic materials made of 100% small metastable tetragonal grains and known as Y-TZP[11].

MECHANICAL PROPERTIES AND AGEING OF ZIRCONIA

Zirconia has mechanical properties similar to those of stainless steel. Its resistance to traction can be as high as 900-1200 MPa and its compression resistance is about 2000 MPa[4]. Cyclical load stresses are also tolerated well by this material. Applying an intermittent force of 28 kN to zirconia substrates, Cales and Stefani found that some 50 billion cycles were necessary to break the samples, but with a force in excess of 90 kN structural failure of the samples occurred after just 15 cycles[19]. Surface treatments can also modify the physical properties of zirconia. One property of zirconia that has not been well studied is the phenomenon of low-temperature degradation or "ageing". Water and nonaqueous solvents can induce the formation of zirconiahydroxides along a crack. This process accelerates expansion of the fracture and can result in reduced strength, toughness, and density, leading to failure of the restoration[14,20-22]. Surface grinding can also reduce strength[23,24]. Kosmac *et al*[15] confirmed this observation and reported reduced mean strength and reliability of zirconium oxide after grinding.

Zirconia is characterized by high flexural strength and fracture toughness as a result of a physical property known as transformation toughening[4,25,26]. The incidence of framework fracture was directly related to the design of the FPD, where inlay retained FPDs (IRFPD) showed the highest failure rate[27,28]. The most common complication observed in zirconia-based restorations was fracture of the veneering porcelain, manifesting clinically as chip-

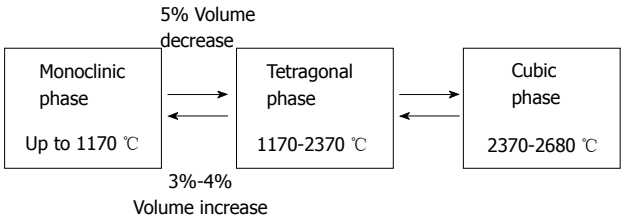

Figure 1 Temperature-related phase transformation of zirconia.

ping fractures of the veneering ceramic with or without exposing the underlying Y-TZP framework[27]. Several factors that may affect the rate of veneering fractures have been investigated. A loss of veneering material may result from an alteration of the crystal structure of the zirconia surface during airborne-particle abrasion of the framework before the veneering process. This may result in a change of the temperature expansion coefficients[15,25]. Other factors include the different surface treatments of the frameworks and the bond strength between the veneering ceramics and zirconia frameworks[29,30].

Sintering a CAD/CAM-milled lithium disilicate layering veneer cap onto the zirconia coping has significantly increased the mechanical strength of crown restorations and represents a cost effective way of fabricating all-ceramic restorations[31]. Milling of new generation full-contour zirconia might be an alternative approach to overcome chipping fractures of veneered zirconia restorations. Fabricating mono-block restorations from pure zirconia could increase the mechanical stability and expand the range of indications[32]. However, no clinical data is available yet.

BIOCOMPATIBILITY OF ZIRCONIA

The biocompatibility of zirconia has been extensively evaluated[4,21,33]. *In vitro* and *in vivo* studies have confirmed the high biocompatibility of Y-TZP with the use of very pure zirconia powders that have been purged of their radioactive content[34-39]. No local (cellular) or systemic adverse reactions to the material were reported[4,11,35,40,41]. Recent studies have demonstrated that fewer bacteria accumulate around Y-TZP than titanium[42-44]. This could possibly be explained by different protein adsorption properties[45]. In terms of periodontal health, none of the studies reported any difference or noted any changes in the biological health of the soft and hard tissues around the zirconia-based restorations. Although some data quantified and explored differences in the biocompatibility of zirconia, no instances of gingival inflammation or periodontitis could be shown[46]. These findings have led to the suggestion that zirconium oxide may be a suitable material for manufacturing implant abutments with a low bacterial colonization potential[44].

Zirconia as implant abutment material was first introduced in 1996[47]. A randomized controlled clinical trial comparing zirconia and titanium abutments supported by 40 single implants was published[48]. After being in function for three years, 18 zirconia and 10 titanium abutments were followed-up. Both abutment materials exhibited survival rates of 100%, as well as similar biological and esthetic outcomes. In an animal study, it was shown that the collagen fiber orientation was similar around zirconia and titanium implant necks. For both materials, the fibers run parallel-oblique and parallel to the implant surface[49]. In a clinical study, a similar degree of plaque accumulation was found at zirconia and titanium abutments at three years. In the same study, when zirconia abutments are used as restoration support, there were no significant differences in bone levels between zirconia and titanium abutments after 3-year follow-up[48].

ESTHETIC PROPERTIES AND LIGHT TRANSMISSION OF ZIRCONIA

All ceramic materials more satisfactorily address the demand for esthetic restorations than metal ceramic restorations with opaque cores[50,51]. However, the translucency of the most durable zirconia-based ceramic crowns is reported to be less than that of lithium disilicate glass ceramics, for which excellent esthetic results are documented[52-56]. In-Ceram Zirconia (VITA Zahnfabrik, Bad Säckingen, Germany), an aluminum oxide-based ceramic with 35% zirconium dioxide, has a relatively low translucency, equal to that of metal ceramic crowns when evaluated using the contrast ratio method[55]. This could be an obstacle to achieving an esthetically acceptable restoration. Among nonzirconia core materials, an optimal esthetic result has been reported with Procera AllCeram (Nobel Biocare AB, Göteborg, Sweden), which is a 99.9% aluminum oxide densely sintered ceramic[57], and IPS Empress (Ivoclar Vivadent AG, Schaan, Liechtenstein) lithium disilicate glass ceramic[58]. The latter evolved in 2005 to IPS e.max Press (Ivoclar Vivadent AG), with improved translucency and mechanical properties[59,60]. Alumina and glass ceramic have, respectively, fair to high relative translucency; nevertheless, their mechanical properties are lower than ZrO_2 ceramics[55,61].

Light transmission through Y-TZP varies as a function of: (1) the composition and thickness of the zirconia framework; and (2) the physical characteristics and degree of glazing of the veneering porcelain[62].

Based on this, the use of zirconia ceramics with different chemical compositions may be significant for clinicians. Additionally, measuring the degree of conversion of different resin luting agents beneath zirconia ceramic materials may produce better clinical outcomes[63]. Future studies should be expanded to include new generation full-contour zirconia[64]. Full-contour zirconia milling blanks are created through a unique patent-pending process. In one process the zirconium oxide powders are milled to further reduce the particle size of zirconium oxide, and mixed with a suitable binder to increase the compaction and density of the green state (compacted powders) and eliminate the closed porosity. The manufacturers claim that, unlike conventional high-pressure

Table 1 Three types of zirconia products and their milling/grinding technology (Information provided by manufacturers)

Milling at green stage (non-sintered)	Cercon base, Cercon (Degudent, Frankfurt, Germany)
	Lava Frame, Lava (3M ESPE, Seefeld, Germany)
	Hint-ELs Zirkon TPZ-G, DigiDent (Girrbach, Pforzheim, Germany)
	ZirkonZahn, Steger (Steger, Brunneck, Italy)
	Xavex G 100 Zirkon, Etkon (Etkon, Grafelfingen, Germany)
Grinding at pre-sintered stage	In-Ceram YZ Cubes, Cerec InLab (Sirona, Bensheim, Germany)
	ZS-Blanks, Everest (KaVo, Leutkirch, Germany)
	Hint-ELs Zirkon TZP-W, DigiDent (Girrbach, Pforzheim, Germany)
	DC-Shrink, Precident DCS (DCS, Allschwil, Switzerland)
	LAVA All-Ceramic System (3M ESPE, Seefeld, Germany)
	Cercon Smart Ceramics (DeguDent, Hanau, Germany)
	Procera Zirconia (Nobel Biocare, Göteborg, Sweden)
Grinding at completely sintered stage	DC-Zirkon, Precident DCS (DCS, Allschwil, Switzerland)
	Z-Blanks, Everest (KaVo, Leutkirch, Germany)
	Zirkon TM, Pro 50, Cynovad (Cynovad, Montreal, Canada)
	Hint-ELs Zirkon TZP-HIP, DigiDent (Girrbach, Pforzheim, Germany)
	HIP Zirkon, Etkon (Etkon, Grafelfingen, Germany)

milling blank manufacture, this processing gives full-contour zirconia improved light transmission, providing a lower, more natural shade value[65].

TYPES OF ZIRCONIA FOR MANUFACTURING PROCEDURE

Three main types of zirconia are available for use in clinical dentistry[66]. Although they are chemically identical, they have slightly different physical properties (*e.g.*, porosity, density, purity, strength), which may or may not be clinically relevant. Zirconia raw material (as previously mentioned) is not a natural product, but is chemically processed from minerals. With cold isostatic pressing, the powders are shaped into ceramic pre-forms. Cold isostatic pressing is the most accepted procedural technique for shaping Y-TZP and produces stable, chalk-like non-sintered green-stage objects with a very high primary density. The green objects are further stabilized and condensed up to about 95% of the theoretical density by means of sintering without pressure in the oxidized atmosphere of a special furnace, forming pre-sintered-type oxide-ceramic blanks[11,67]. Additional compression can be achieved with Hot Isostatic Postcompaction (HIP) performed at 1000 bar and 50 ℃ below the sintering temperature[67]. This procedure removes residual porosity and produces dense, fully-sintered-type oxide-ceramic blanks. Carrying out HIP on Y-TZP results in a gray-black material that usually requires subsequent heat treatment to oxidize and restore whiteness[68].

Zirconia ceramics are used in dentistry as materials for frameworks, generally fabricated by means of milling the zirconia block using a CAD/CAM machine system[69-74]. Blocks can be milled either at the green stage, the pre-sintered stage or the completely sintered stage. Green-stage zirconia blocks can be milled using dry carbide burs, pre-sintered zirconia blocks can be milled using carbide burs under cooling liquid, and milling of completely sintered zirconia blocks requires the use of diamonds under cooling liquid[75]. The three available types of zirconia products are shown in Table 1 together with the milling/grinding technology used in each case.

Frameworks made from green and pre-sintered zirconia are milled in an enlarged form to compensate for the shrinkage that occurs during sintering, usually 20%-25% for a partially-sintered framework[76]. The milling process is faster and the wear and tear on hardware is less than when milling from a fully-sintered blank. The framework is subsequently post-sintered in special furnaces (at about 1500 ℃) to reach the fully-sintered stage. The color of the zirconia can be individualized with the addition of oxides to the green-stage framework[68].

The question often arises as to which type of zirconia is best to use. It appears that each has advantages and disadvantages. Fully-sintered HIP zirconia has a denser polycrystalline structure with less porosity than non-HIP material, and this should translate clinically into increased resistance to fracture[77]. On the other hand, some investigators have questioned whether the amount of grinding needed during milling of fully sintered zirconia and the heat that is generated, cause surface and structural defects that can have adverse clinical implications[78]. The marginal fit of either type of material, however, is associated with very acceptable clinical results. Margin fitting of milled zirconia is as good as, if not superior to the fit of a restoration fabricated from a high noble alloy. Studies have measured the marginal gap of CAD/CAM-milled zirconia of both varieties and found it to be 40 to 70 μm[79]. However, compared to the alternative method, milling of fully sintered zirconia blocks is a time consuming process that causes greater wear of the diamond burs and is more expensive. Hence, from that point of view, green-stage zirconia could be regarded as more advantageous[67].

BONDING TO ZIRCONIA

The longevity of an indirect restoration is closely related to the integrity of the cement at the margin[80]. Although the use of zirconia ceramics for dental applications is

ongoing, the best method to achieve a durable bond between the ceramic and the tooth structure is still unknown[81]. The only consensus found in the literature is that hydrofluoric acid etching and common silane agents are not effective with zirconia ceramics[81-83].

Several studies have investigated the bond strength and the durability of various bonding methods used to form high-strength zirconia ceramics. One technique commonly used to condition the ceramic surface is that of air abrasion[77,84-86]. Air abrasion with aliminium oxide particles is routinely performed to remove layers of contaminants, thus increasing micromechanical retention between the resin cement and the restoration[80,87,88]. These particles may or may not be silica-coated (with tribochemical treatment)[89-91].

Other techniques for the superficial treatment of zirconia ceramics which have been investigated are laser, plasma spraying and fusing glass pearls to the zirconia surface[92,93]. Higher laser power settings (400-600 mJ) cause excessive material deterioration, making them unsuitable as treatments for zirconia surfaces. Irradiation with 200 mJ provides mild surface alterations, with features intermediate between the effects of air abrasion and higher laser intensities[92]. Plasma spraying and glass pearl fusion treatments were found to improve the bond strength of resin cements to the surface. However, they were not compared with conventional methods of surface treatments for Y-TZP ceramics, such as air abrasion and tribochemical coating[93].

In other studies several coating agents were used to enhance the formation of chemical bonding with zirconia but only those agents that contain a phosphate monomer agent were effective in establishing a reliable bond with zirconia materials[84,94].

A recent study focusing on the long-term stability of zirconia resin bonding shows that it is directly related to the chemistry of the materials used, including primers. The authors suggest that a more hydrophobic compound is required to better resist the detrimental effect of hydrolysis in order to gain full benefit from the primers[95-97].

In a novel approach to enhance zirconia resin bond strength, selective infiltration-etching of zirconia-based materials has been tried. This method creates a retentive surface where the adhesive resin can infiltrate and interlock in order to establish a strong and a durable bond with zirconia[98-101].

CONCLUSION

Several positive characteristics of zirconia, such as biocompatibility, color and mechanical properties, make the material suitable for use in modern dentistry. However, ceramic bonding, ageing, light transmission and manufacturing processes are all factors that need to be further evaluated in order to guide the successful use of zirconia as a prosthetic restorative material. Milling zirconia to full-contour might be an alternative to traditionally veneered restorations.

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ORIGINAL ARTICLE

Clinical evaluation of implants in patients with maxillofacial defects

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Abstract

AIM: To show the efficacy of reconstruction and rehabilitation of large acquired maxillofacial defects due to tumor resections and firearm injuries.

METHODS: The study group comprised of 16 patients (10 men and 6 women) who were operated on because of their maxillofacial defects under local and general anesthesia between June 2007 and June 2011. Prosthetic treatment with the aid of dental implants was performed for all of the patients. Eight patients received an implant supported fixed prosthesis; six patients received implant supported overdentures and two patients received both. Patients were followed up postoperatively for 1 to 4 years. Implant success and survival rates were recorded. Panoramic radiographs were taken preoperatively, immediately after surgery, immediately after loading and at every recall session. Peri-implant and prosthetic complications were recorded. Subjects were asked to grade their oral health satisfaction after treatment according to 100 mm visual analog scale (VAS) and the oral health related quality of life of the patients was measured with the short-form Oral Health Impact Profile.

RESULTS: Five implants (3 in the mandible, 2 in the maxilla) in five patients were lost, while the other 53 survived, which brings an overall survival rate of 91.37% on the implant basis, but 68.75% on patient basis. All the failed implants were lost before abutment connection and were therefore regarded as early failures. For all failed implants, new implants were placed after a 2 mo period and the planning was maintained. The mean marginal bone loss (MBL) was 1.4 mm on the mesial side and 1.6 mm on the distal side of the implants. Five of the implants showed MBL > 2 mm (mean MBL = 2.3 mm) but less than 1/2 of the implant bodies and therefore were regarded as not successful but surviving implants. The VAS General Comfort mean score was 85.07, the VAS Speech mean score was 75.25 and the VAS Esthetics mean score was 82.74. No patient reported low scores (score lower than 50) of satisfaction in any of the evaluated factors. The mean of OHIP-14 scores was 5.5.

CONCLUSION: Although further follow up and larger case numbers will give more information about the success of dental implants as a treatment modality in maxillofacial defects patients, the actual results are encouraging and can be recommended for similar cases.

Key words: Dental implant; Maxillofacial defect; Overdenture; Prosthesis; Visual analog scale; Marginal bone loss

Core tip: Dental implant treatment is efficient in the reconstruction and rehabilitation of large acquired maxillofacial defects due to tumor resections and firearm injuries. Although further follow up and larger case numbers will give more information about the success of dental implants as a treatment modality in patients

with maxillofacial defects, the actual results are encouraging and can be recommended for similar cases.

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INTRODUCTION

Maxillofacial defects are initiated either by trauma or tumor resection. In both cases, the function and esthetics of the patients are impaired and a prosthetic rehabilitation is essential. Since removable prosthetic appliances function on soft tissues and the denture bearing areas are supposed to be composed of keratinized mucosa, defect cases create a challenge. Most of the acquired defects are surgically covered with thin mucosa which is not able to support denture bases. In this manner, dental implant treatment is a valuable aid to support the dentures, leaving the non-keratinized mucosa unloaded[1]. The use of dental implants in patients after trauma due to oral surgical resections, deformities, accidents or firearm injuries can give patients better function and self confidence by the achievement of retention and stability[1,2].

The structural and functional rehabilitation of maxillofacial defects, after oral tumor resection, maxillofacial trauma such as firearm injuries, avascular bone necrosis or large bone cysts, requires prosthetic reconstruction in most of the related patients. Local oral conditions, general health, as well as psychological, social and economic aspects, determine the final treatment outcome of the prosthetic rehabilitation[3]. The prosthodontic treatment in these patients creates a challenge due to several factors, such as bone volume deficiency, low quality of bone, altered anatomy, xerostomia, missing attached gingiva and associated fragile mucosa[4,5].

Maxillofacial defects caused by different reasons represent a challenging problem with regard to restoring optimal oral function and esthetics. These kinds of wounds exhibit a spectrum of complexity and mostly include extensive soft tissue trauma complicated by burns, foreign bodies, fractures and/or tissue loss. Since the clinician often faces situations with a remarkable tissue loss, dental implants are crucial to secure retention of the prosthetic appliances. Meanwhile, it is well known that dental implants enhance patient satisfaction and quality of life[6], provide improved retention and stability and enhanced chewing function and have the potential to preserve substantial bone[7-9].

The aim of this study was to report the treatment outcome of patients up to 4 years after reconstruction of oral and maxillofacial defects with a dental implant supported prosthesis and focus on prosthetic aspects, implant survival/success, patient satisfaction and quality of life.

MATERIAL AND METHODS

Patient recruitment, clinical and radiographic procedures

Fifty-eight implants placed in 16 patients with maxillofacial defects caused either by trauma, such as firearm injuries or accidents, or tumor resections of oral cancers at a university clinic between June 2007 and June 2011 were included in the present study. Informed written consent with regard to treatment and measurement procedures was given by all patients and approval from the university ethics commission was duly obtained. All the implants came from one manufacturer (Straumann®, Basel, Switzerland) and were placed by the same oral and maxillofacial surgeon.

All the patients suffered from alterations of the oral cavity (Table 1). Seven out of 16 patients (6 male, 1 female) had limitations in jaw opening (microstomia). The alterations were due to firearm injuries (3 patients: 2 male, 1 female) or ablative tumor surgery (13 patients: 8 male, 5 female) (Figures 1A-C). The details of the patients are presented in Table 1. For the patients with firearm injuries (n = 3; Figure 2A and B), the implant treatments were performed 1 year after reconstructive surgeries for the patients with firearm injuries and 2 years after the radiotherapy and/or chemotherapy for the patients who had undergone ablative tumor surgeries.

Surgery was performed as recommended by the manufacturer, using a one-stage surgical protocol in 10 patients (Figure 1D) and a two-stage surgical protocol in 6 patients. In all of the patients, large bony reconstructions were carried out by using free monocortico-cancellous iliac bone grafts or vascularized tissue flaps.

Prosthetic treatment of the defect patients was performed by 2 prosthodontists with 10 years of clinical experience. After implant surgery, 3 mo for the lower jaw and 6 mo for the upper jaw, osseointegration was waited for and then 8 patients received an implant supported fixed prosthesis (Figures 1E and 2A); six received implant supported overdentures (Figure 1B) and 2 received both (Table 1). The chosen prosthetic superstructures of the patients are presented in Table 1.

All participants received digital (Morita Veraview IC5®, J Morita MFG Corp, Kyoto, Japan) or analog panoramic radiographs (Planmeca®, Proline XC, Helsinki, Finland) using the imaging equipment before the surgery for treatment planning, immediately after and every year after loading of the implants for the evaluation of marginal bone levels of the implants.

Recalls were routinely performed 12, 24, 36 and 48 mo after loading. At each recall session, a clinical examination was performed by the same examiner. Implant success and survival rates were determined based on the following criteria: implants fulfilling all of the following criteria were regarded as successful[10]: no pain or tenderness upon function; 0 mobility (checked by manual manipulation); < 2 mm radiographic bone loss from initial surgery; no exudate history.

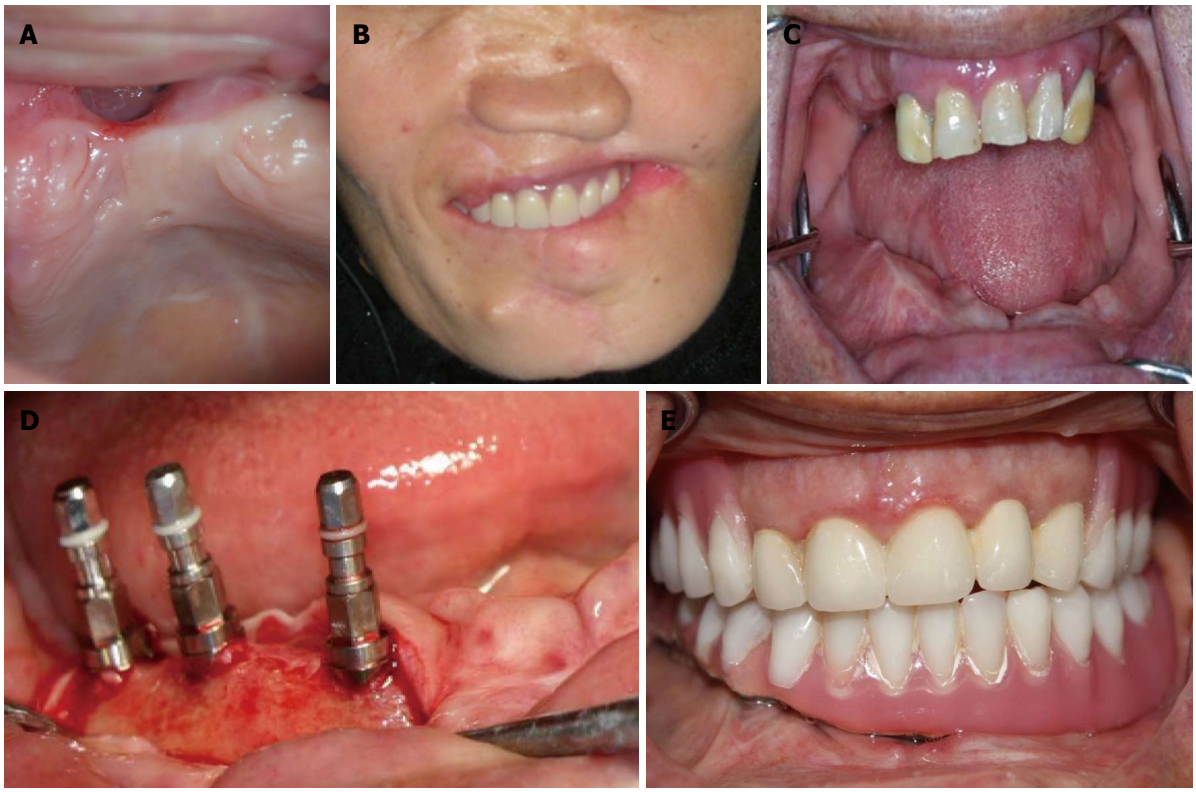

Figure 1 Intraoral view of a patient. A: Intraoral view of a patient after reconstruction of a gunshot wound; B: Delivered maxillary overdenture of the patient with the gunshot wound; C: Intraoral view of a patient after ablative tumor surgery; D: Insertion of dental implants using one-stage surgical protocol; E: Intraoral view of implant supported fixed prosthesis.

Table 1 Details of patients and implants

Patients (*n*)	16
Implants (*n*)	58
Patient age (mean, yr)	39
Patient gender	10 female, 6 male
Type of injury	firearm injuries (3 patients; 2 male, 1 female) or ablative tumor surgery (13 patients; 8 male, 5 female)
Insertion time of the implants	1 year after reconstructive surgeries for firearm injuries (*n* = 3) 2 years later for the patients who have undergone ablative tumor surgery (*n* = 13)
Loading time of the implants	3 mo after insertion for lower jaw and 6 mo after insertion for upper jaw for every patient
Location of implants	41 in the mandible, 17 in the maxilla
Type of prosthesis	8 patients received fixed prosthesis, 6 patients received overdentures, 2 patients received both

Implants with at least one of the following criteria but with no mobility (checked by manual manipulation) were regarded as surviving but not successful[10]: may have sensitivity on function; radiographic bone loss > 2 mm but less than 1/2 of implant body; may have exudate history.

Radiographic evaluation and bone level assessment

Panoramic radiographs were taken preoperatively (Figure 2B), immediately after surgery (Figure 2C), immediately after loading (Figure 2D) and at every recall session. In cases of insufficient quality, intraoral radiographs were taken as well. Mesial and distal marginal bone levels of all implants were determined at baseline and recall evaluations. The analog panoramic radiographs were scanned and digitized (Epson 1680 Pro®, Seiko Epson Cooperation, Nagano, Japan). Measurements were obtained from images of successive radiographs, which were analyzed at X20 magnification with the use of a software program (CorelDraw 11.0®, Corel Corp and Coral Ltd, Ottawa, Canada).

The known diameter of the implant at the collar region according to the manufacturer's dimensions of the respective implants was used as a reference point[11]. The distance from the supracrestal widest part of the implant to the crestal bone level was measured on the magnified images. To account for variability, the implant dimension (width) was measured and compared with the documentation dimensions; ratios were calculated to adjust for distortion. Bone levels were determined by applying a distortion coefficient (true bone height is equal to true implant width multiplied by bone height as measured on the radiograph, which is then divided by the implant diameter measured on the radiograph). The actual bone level measurement was performed independently by 2 examiners (a prosthodontist and an oral and maxillofacial surgeon) who were calibrated before the study.

The average from the 2 examiner calculations was used as the marginal bone level value. The level at which the marginal bone seemed to be attached was assessed by visual evaluation at the distal and mesial surfaces of all implants.

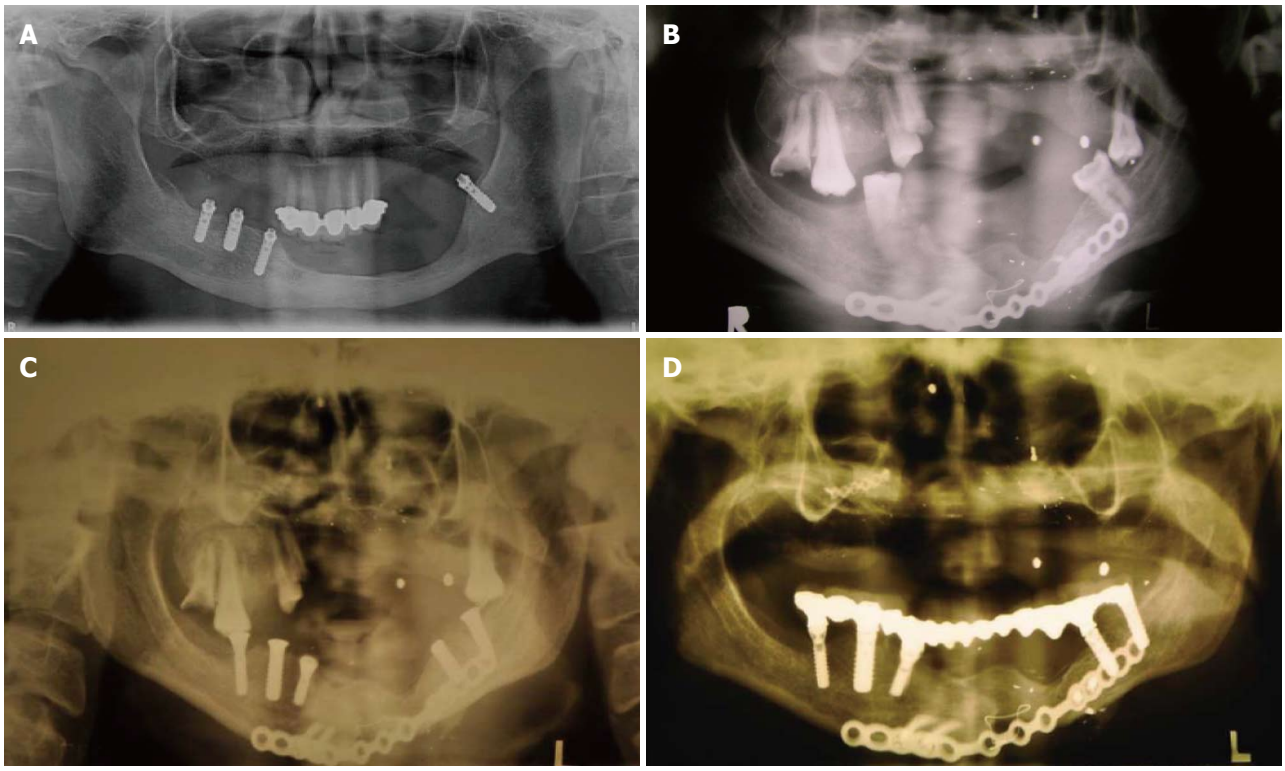

Figure 2 Panoramic radiograph. A: Panoramic radiograph taken after implant surgery; B: Panoramic radiograph taken before implant surgery; C: Panoramic radiograph taken after implant surgery; D: Panoramic radiograph taken after loading.

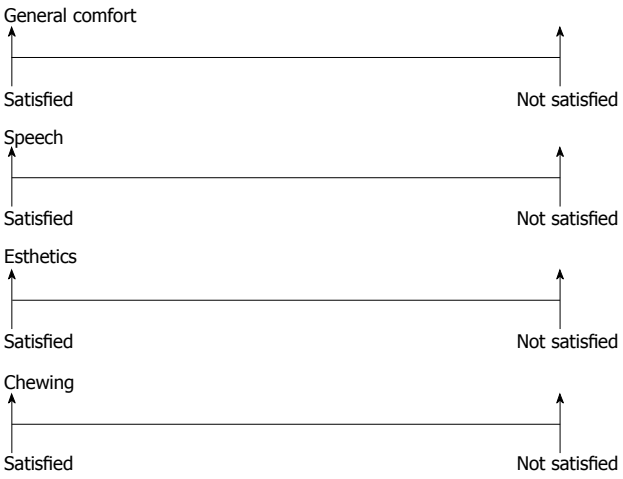

Figure 3 The visual analog scale form for general comfort, speech, esthetics and chewing.

Patient satisfaction and oral health related quality of life outcomes

Subjects were asked to grade their oral health satisfaction after treatment on a 0-100 mm visual analog scale (VAS) for 4 separate factors: general comfort, speech, esthetics and chewing (Figure 3). The scales were anchored by the extremes of potential responses (*e.g.*, completely satisfied-completely dissatisfied: the higher the score, the more satisfied the subject).

For the determination of quality of life of the patients, all subjects were asked to complete the Turkish version of the short-form Oral Health Impact Profile (OHIP-14), which has previously been determined to be valid and reliable[12]. Subjects rated each of the 14 items on a 5-point Likert scale from 0 = "never" to 4 = "very often". Items were added up to yield the total score. Achievable OHIP-14 score ranged from 0-56, with lower scores representing higher oral health-related quality of life[13].

RESULTS

Implant success, survival and failures

Five implants (3 in the mandible, 2 in the maxilla) in five patients were lost, while the other 53 survived, which brings an overall survival rate of 91.37% on the implant basis and 68.75% on a patient basis. Out of the 53 surviving implants, 48 were regarded as successful according to the criteria proposed by Misch *et al*[10] and thus the success rate was calculated as 82.75%. All the failed implants were lost before abutment connection and therefore regarded as early failures [14]. For all failed implants, new implants were placed after a 2 mo period and the planning was maintained.

Peri-implant complications and marginal bone loss

The mean marginal bone loss (MBL) was 1.4 mm on the mesial side and 1.6 mm on the distal side of the implants. 5 of the implants showed MBL > 2 mm (mean MBL = 2.3 mm) but less than 1/2 of implant bodies and were therefore regarded as not successful but surviving implants.

The MBL on the distal and mesial aspects of the implants up to 48 mo following loading did not exceed 2

Table 2 Oral HEALTH IMPACT PROFILE total and 7 domain mean scores

OHIP total	5.5 (range 0-56)
Functional limitation	0.31(range 0-8)
Physical pain	1.56 (range 0-8)
Psychological discomfort	1.37 (range 0-8)
Physical disability	1.06 (range 0-8)
Psychological disability	0.56 (range 0-8)
Social disability	0.18 (range 0-8)
Handicap	0.25 (range 0-8)

OHIP: Oral Health Impact Profile.

mm on average.

In two cases using fixed-detachable (hybrid type) restorations, excessive soft tissue under the prosthesis were observed at the 12 month recall appointment. For treatment, the hybrid dentures were unscrewed and removed and the large hyperplasic tissues were surgically excised. In order not to cause further trauma, the borders of the denture bases were adequately shortened in these areas and a week after surgical intervention, hybrid dentures were screwed to the abutments and tightened with the appropriate torque wrenches.

Prosthetic complications

During the observation period of up to 48 mo, the following prosthetic complications occurred: 1 fracture of a mandibular hybrid denture; 1 fracture of an abutment screw of a locator abutment; 1 fracture of the male part of a ball abutment; the requirement of rebasing in two overdentures (1 in the maxilla, 1 in the mandible); chipping of the veneering of a hybrid denture; and the requirement of substitution of the retention mechanism of 2 overdentures after an average service period of 21 mo (9-28 mo).

All prosthetic complications were eliminated and repaired; the fractured mandibular hybrid denture was redone on a new impression and model. Two overdentures were relined and the two fractured abutments were replaced. The chipped part of the hybrid denture was repaired and the retention mechanisms of the overdentures were replaced.

Patient satisfaction and oral health related quality of life scores

Patient satisfaction scores were as follows: VAS General Comfort mean score = 85.07 out of 100; VAS Speech mean score = 75.25 out of 100; VAS Esthetics mean score = 82.74 out of 100. No patient reported low scores (score lower than 50) of satisfaction in any of the evaluated factors. The mean of OHIP-14 scores was 5.5. The OHIP-14 total and the 7 domain scores of the patients are presented in Table 2.

DISCUSSION

Implant-supported prostheses for maxillofacial defect patients have become a reliable treatment modality[1,2]. It may be expected that in this kind of patients, implant failures increase since the conditions are tougher compared to conventionally placed and loaded dental implants. Often the implants are facing situations such as altered anatomy, xerostomia, missing attached gingiva around the implant neck or inconvenient bone[15-17]. It should be pointed out that maintenance of daily hygiene is very important for these patients, especially for patients suffering from xerostomia. With the absence or presence of small amounts of saliva, the oral cavity becomes more prone to oral infections; thus, the risk of implant failures may rise. As shown in one of our cases, the long edentulous span, which cannot be covered by a denture base because of grafted skin covering the reconstruction, had to be restored with a hybrid denture supported by a few implants (Figure 1C-E). Additionally, missing attached gingiva is known to be a disadvantageous condition for peri-implant health. In the present clinical study, the implant survival rate and success was lower compared to implants in conventional sites. In spite of a higher implant failure rate, this treatment gradually became a well-accepted option in the therapeutic spectrum of oral and maxillofacial deformities[18,19]. In spite of the improper implant positions in several cases, a success rate of 82.75% was obtained. Due to the need of malpositioning of the implants in the remaining tissue support, it could be expected that the survival and success rate of these implants would be impaired. There are studies reporting that implants had comparable success rates when they are placed angled or malpositioned[20]. The implant success and survival rates in the present study showed similarities to the studies illustrating the successful use of osseointegrated implants in the reconstruction of traumatic craniomaxillofacial injuries and in the rehabilitation of oral function in head and neck cancer patients[5,21-23]. However, the present study showed a higher rate of implant failure, peri-implant soft tissue complications and marginal bone loss than studies showing the implant data of patients without maxillofacial defects[6,8,11-14]. On the basis of clinical observations, bone loss ranging between 1 and 2.6 mm has been reported to occur around the margin of successfully osseointegrated dental implants[24,25]. In spite of a lack of consensus, the values generally accepted as a reasonable guideline for bone loss since the late 1980s is 1.5 mm for the first year after loading the implants and 0.2 mm of additional loss for each following year[10,26].

Regarding this guideline, the marginal bone loss rate reported here in the present study could be accepted as successful in spite of unfavorable conditions. On the other hand, it should be noted that the marginal bone loss rate presented in more recent studies lies much lower. The minimization of crestal bone loss was explained by surface roughness, evaluated as one of the key factors[27]. Nevertheless, the patients' clear judgment in favor of dental implant supported prosthetic rehabilitation in this study, which encourages this treatment modality. In the present study, a high level of patient satisfaction and quality of life were achieved (Table 2). The obtained

VAS and quality of life scores in this pilot study show similarities to the study of Schoen *et al*[21] which investigated the patient satisfaction and quality of life outcome of implant treatment in head and neck cancer patients[1]. Additionally, our results are comparable to other studies concerning treatment with dental implants[6,8,28-30].

In the present study, the patients were not asked to complete the VAS and OHIP-14 questionnaires before the treatment; thus, it was not possible to compare the pre and post treatment scores, which may be regarded as a limitation. All the patients were unable to function with the pre-treatment oral conditions; therefore, the authors did not consider it necessary and moral to constrain the patients in completing the questionnaires before treatment. Additionally, in the opinion of the authors, the OHIP-14 questionnaire is very hard to comprehend and could cause misleading results in these patients. The form could be modified for patients with maxillofacial defects just like the previously made modification for edentulous patients as OHIP-EDENT[31].

Early management of injured patients must focus on the basics of resuscitation. The secondary target in the treatment of these cases, however, should focus on tissue preservation, abstaining from unnecessary tissue resection, because the placement of dental implants can be problematic from time to time. The attention paid at the early stage of intervention can have an important impact on the quality of life of patients.

As a general approach at the dental school, the implant treatment has to be postponed for a certain period if a major resection and reconstruction has been performed. If radiotherapy and/or chemotherapy is administered, the patient has to wait at least 2 years for the implantation, as suggested previously[5]. The prosthetic complications recorded in the present study were slightly over the average of prosthetic patients treated in the related university clinic. Although complications, such as requirement of rebasing, chipping of veneering material and substitution of retention mechanism, are routinely encountered and well documented in the literature[32], the fracture of a hybrid denture, a locator abutment or of the male part of a ball attachment is not common. The misalignment or strategically disadvantageous numbers and positions of implants may be a factor that explains higher rates of complications in the present patient group.

Oral rehabilitation becomes even more complicated with the presence of microstomia[33], which can be encountered in this kind of patients. Microstomic patients experience considerable limitation in jaw opening and overall jaw mobility. This limitation in the oral opening makes gaining access to the oral cavity difficult, depending on the severity of microstomia. Therefore, traditional approaches for dental restoration should be modified to accommodate microstomia. Various treatment approaches have been proposed for microstomic patients, with or without endosseous implants. Reduced mouth opening may prevent instruments from safely entering the mouth for insertion of the implants. This is a critical factor in determining whether implant treatment can be provided and in deciding the number of inserts needed and the best places for insertion[34].

In the present study, 3 patients had a limited intraoral access, requiring modification of the approach. Also, there might be problems with the precision of dental laboratory work because of the inaccurate impressions which were hardly made with the modification methods[33]. Therefore, the precision of fit of the dental frameworks were very limited (Figure 2D). The strains due to the misfit of the denture can be a reason for the failures and prosthetic complications. In cases of firearm injuries, the severity of the defect resulting from facial firearm injuries varies according to the caliber of the weapon used, the distance from which the patient is shot and the part of the body involved[35]. Close range, high velocity firearm wounds can result in devastating functional and esthetic consequences. Maxillofacial traumas are mostly encountered in males (78%) and at a higher rate between the ages of 20-39 years. There are many reasons for maxillofacial trauma, such as fighting (48.2%), falling (26.2%), car accidents (4.2%) and firearm injury (1.2%)[36,37]. The epidemiology of facial fractures varies in type, severity and cause, depending on the population studied[38]. The differences between populations in the causes of maxillofacial fractures may be the result of risk factors and cultural differences between countries but are more likely to be influenced by the injury severity[39].

In situations with insufficient bone volume, invasive surgical procedures such as maxillary sinus floor elevation or the zygomatic implant placement[19], procedures mainly accomplished by maxillofacial surgeons, can be an alternative. However, individuals of the related patient group could appeal against additional complex surgical interventions after the long and griping procedures they have endured.

Meanwhile, it is a well known fact that the first year is critical for implant failure and for the largest portion of marginal bone loss around dental implants[34]. The results of an investigation showed that practically all implant losses occurred during the first 2 years, whereupon a steady state seemed to follow for up to 5 years after loading[40].

Despite disadvantageous loading conditions and poor bone quality and quantity, all the presented cases showed a stable situation around the implants after a period of 12-48 mo of loading time. Although further follow up and larger case numbers will give more information about the success of dental implants as a treatment modality in maxillofacial defect patients, the actual results are encouraging and can be recommended for similar cases. Even although the success and survival rate is slightly lower than conventionally loaded implants due to tougher conditions, dental implants seem to be a valuable aid in the maintenance of comfortable rehabilitation of maxillofacial defect patients.

COMMENTS

Background

In patients with maxillofacial defects, implant failures may increase, since the conditions are harder compared to conventionally placed dental implants. Often

the implants are facing situations such as altered anatomy, xerostomia, missing attached gingiva around the implant neck or inconvenient bone.

Research frontiers

The treatment outcome of patients with maxillofacial defects up to 4 years after dental implant supported prosthesis should be investigated and prosthetic aspects, implant survival/success, patient satisfaction and quality of life of these patients should be demonstrated. In this study, the authors show that dental implants seem to be a valuable aid in the maintenance of comfortable rehabilitation of maxillofacial defect patients.

Innovations and breakthroughs

Studies of patients with maxillofacial defects are mostly case reports. This is one of the first studies to report the outcome of dental implant treatment in these patients.

Applications

The actual results are encouraging and dental implant treatment can be recommended for similar cases.

Peer review

The authors examined the prosthetic and peri-implant complications, patient satisfaction, marginal bone loss and success and survival of implants in patients with maxillofacial defects. The obtained positive results will be a valuable guide for clinicians facing the same difficulties in patients.

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BRIEF ARTICLE

Evaluation of factors affecting the success rate of orthodontic mini-implants by survival analysis

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Abstract

AIM: To investigate the success rate of mini-implants and its characteristics and risk factors by survival analyses.

METHODS: Three hundred and ninety-four mini-implants of the same type were placed by a single clinician. Age, gender, treatment duration, time of failure, side and jaw of implantation and the soft tissue at placement site were recorded. Odds ratio, survival curves, and Cox proportional hazard model were applied to evaluate the factors influencing the mini-implants' success rate.

RESULTS: The cumulative success rate was 88.1%. The maxilla had a significantly higher success rate than that of the mandible (91.7% *vs* 83.7%, respectively, $P = 0.019$). Placement of mini-implants in the attached gingiva (AG) showed a higher success rate than that of the mucogingival junction (MGJ) and mucous membrane (MM) (AG, 94.3%; MGJ, 85.8%; MM, 79.4%; $P < 0.001$). Significant association was found between the jaw and the gingival tissue type ($P < 0.001$). There were no significant differences between maxilla and mandible when compared within each placement site.

CONCLUSION: The gingival tissue type had the most significant effect on the success rate of the mini-implant with higher success rate in the attached gingiva.

Key words: Mini-implant; Success rate; Survival analysis; Gingival tissue; Treatment planning

Core tip: Anchorage reinforcement is a critical factor for successful orthodontic treatment outcome. Mini-implants are applied to achieve various dental movements such as anterior retraction, molar protraction and distalization, intrusion, extrusion, and correction of midline and occlusal canting. The gingival tissue type had the most significant effect on the success rate of the mini-implant with higher success rate in the attached gingiva.

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INTRODUCTION

Anchorage reinforcement is a critical factor for successful orthodontic treatment outcome. Mini-implants are

applied to achieve various dental movements such as anterior retraction, molar protraction and distalization, intrusion, extrusion, and correction of midline and occlusal canting[1-4].

The factors affecting the success rate of mini-implants have been investigated extensively but not all of them are agreed upon regarding their significance by the investigators[5-10]. Type of mini-implant was suggested as a contributor to the success rate[11,12]. Age and gender of patients, the jaw and side receiving the mini-implant, and the type of gingival tissues were not significantly associated with the success rate[8,11,13,14].

However, Lee *et al*[15] reported a significant effect of age, and Manni *et al*[12] demonstrated the gender as a significant factor. In addition, peri-implant soft tissue characteristics may be a contributing factor[16]. Moon *et al*[13] also reported significant differences between placement sites between different teeth. Also, vertical skeletal pattern was reported to influence the success rate[10].

Recently, three or more types of mini-implants were placed by more than one operator[10,11,14,16]. However, Lee *et al*[15] inserted a single type of mini-implants and reported that there are no significant differences in the success rate according to clinicians. In Park *et al*[17] the mini-implants were placed by one clinician, but the sample size was relatively small for both reports.

However, a well-controlled study with larger sample size of a single type of mini-implants placed by one experienced clinician has not been conducted. This can minimize the effect of the operator- and mini-implant-related factors on the evaluation of success rate.

Therefore, the purpose of this study was to investigate the success rate of mini-implants and its characteristics and risk factors using the same type of mini-implants placed by single clinician by survival analyses.

MATERIALS AND METHODS

A hundred and sixty four patients (47 male, 117 female; mean age 24.0 ± 6.8 years) treated with fixed appliance from July, 2009 to March, 2010 in a private orthodontic clinic were included in this retrospective study. Those who had special medical history such as osteoporosis, thyroid problem, diabetes, and hypertension were excluded.

A total number of 394 mini-implants were placed for anchorage reinforcement by one right-handed experienced clinician using a single placement technique (30° to the surface of soft tissue and about 20 N•cm torque on the self drilling miniscrew) and were loaded 3 wk after placement with a similar amount of force. Only one type of mini-implants was used to exclude the effect of the screw material and design (6.0 mm in length and 1.5 mm in diameter, Biomaterials Korea, Seoul, Korea).

The records were examined to retrieve the following data: age, gender, date of mini-implant placement, date of failure (if occurred), date of removal at the end of treatment, location (upper, lower, right, left) and gingival tissue type at placement site [attached gingiva (AG), mucogingival junction (MGJ), mucous membrane (MM)]. The success of the mini-implant was defined as being functionally stable till the end of the treatment without signs of inflammation. Meanwhile, failure was recorded in case of removal of the mini-implant due to looseness.

Statistical analysis

SAS 9.2 (SAS Institute Inc. Cary, NC, United States) was used for the statistical analysis. The Fisher exact test significance and odds ratio statistics were calculated. A nonparametric life table method was used to easily visualize the hazard function over time. Association between significant variables was assessed by χ^2 test. Kaplan-Meier survival curves were generated, and the Gehan generalized Wilcoxon test was used to identify the variables associated with implant failure. Prognostic variables associated with implant failure were identified with the Cox proportional hazard model which is a survival model that relate the time passed before an event happens to one or more covariates (in our study: age, gender, jaw, side, and gingival tissue) that might be associated with that quantity of time. The level of statistical significance was set at 5%.

RESULTS

There was no significant difference in the success rates between implantation sides, gender, and age. However, there were significant differences between upper and lower implantation (91.7% *vs* 83.7%, respectively, $P = 0.019$) and according to the gingival tissue type at the placement site (AG, 94.3%; MGJ, 85.8%; MM, 79.4%; $P < 0.001$) (Table 1).

The hazard function of mini-implant survival time was regarded as the instantaneous failure rate[18]. As the latest failure event was at 27 wk, the function showed that the risk of failure was highest immediately after placement and then decreased to zero till the end of the treatment. The linear fit of the hazard function was $R^2 = 0.62$ with a negative slope over time (Figure 1).

The Kaplan-Meier survival curve according to jaw and gingival tissue type (Figure 2) demonstrated high success rates for all subgroups. The Gehan generalized Wilcoxon test revealed that the implants placed in the maxilla had a higher success rate than those placed in the mandible ($P = 0.014$). Also, those placed in the attached gingiva had a significantly higher survival rate than other subgroups ($P < 0.001$).

χ^2 test verified a significant association between the jaw and the gingival tissue type ($P < 0.001$) (Table 2). By Fisher's exact test and odds ratio analysis, there were no significant differences between maxilla and mandible when compared according to gingival tissue type, independently (Table 3). The Cox proportional hazard model also showed that the gender and gingival tissue type are significant factors for mini-implant survival (Table 4). The estimated probability of failure was lower for females ($P < 0.001$) and the attached gingiva ($P = 0.019$).

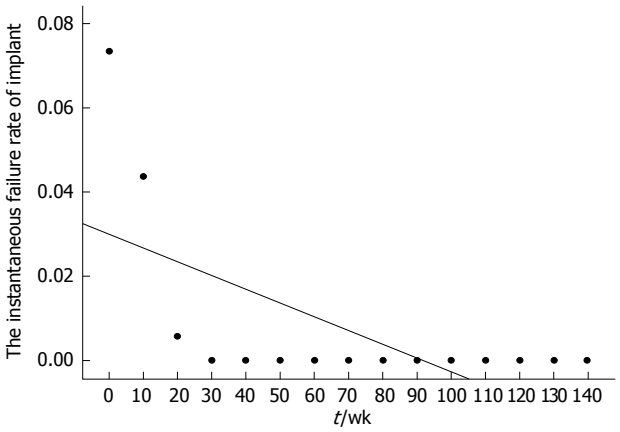

Figure 1 Instantaneous failure curve of mini-implant: The hazard function shows that the maximum risk is immediately after mini-implant placement and then it declines to zero by time. The linear fit of the hazard function was $R^2 = 0.623$.

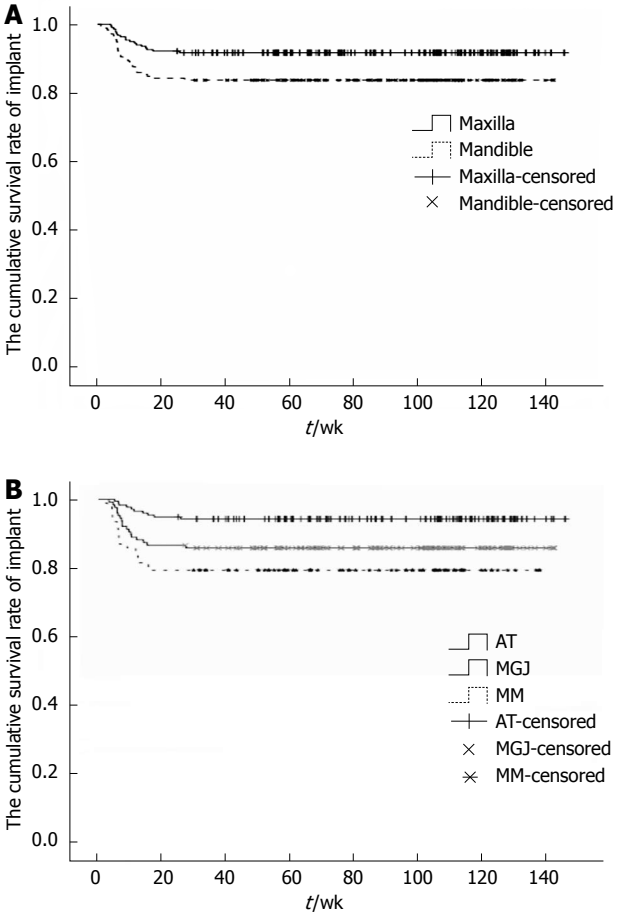

Figure 2 Kaplan-Meier survival analysis of mini-implant by jaw and gingival tissue. A: The survival rate of mini-implants placed in the maxilla was significantly higher than that of those placed in the mandible; B: The survival rate of mini-implants placed in the attached gingival (AG) was significantly higher than that of those placed in the mucogingival junction (MGJ) and mucous membrane (MM). The duration of survival in the censored cases was measured from mini-implant placement to completion of treatment.

DISCUSSION

With improvement of mini-implant materials, design and placement technique, recent studies have often reported mini-implant success rates higher than 90%[15,19]. On the other hand, since it is rare that a patient receives only one mini-implant during orthodontic treatment, the success rate faced by clinicians throughout treatment may be substantially lower due to presence of multiple mini-implants in each patient.

In our study, the success rate (88.1%) was slightly lower than that in Lee *et al*[15] (91.5%), higher than Manni *et al*[12] (81%) and similar to Cheng *et al*[16] (89%). Moreover, several studies evaluated numerous factors affecting the success rate of mini-implants[5,14,17,20]. However, most of them assessed many heterogenic variables using a small sample size that increase type II errors and decrease statistical power. In our study, to eliminate the factors related to the clinician and the mini-implant, only one clinician placed 394 mini-implant of the same type following the same insertion technique.

Recently, Manni *et al*[12] evaluated 12 different factors affecting the stability of mini-implants. Although the mini-implants in their study were placed by the same clinician, they were of 3 different types. Furthermore, the evaluation of too many variables may lead to generation of higher-order interactions resulting in a complicated result interpretation[21]. Our research was limited to only five host variables to avoid such a complication.

Lee *et al*[15] also evaluated five variables affecting the success rate of the mini-implants. However, the anatomical location and the soft tissue of the insertion site were not included in their study. They found patient's age to be the only significant factor that affects the success rate of mini-implants. They recommended special caution when planning mini-implants for young patients. On the contrary, our results showed that the age was not a significant factor in determining the success of mini-implants.

Several reports demonstrated a significant effect for age on the success rate of mini-implants[15,20]. The higher risk of failure in younger patients could be attributed to their lower bone density[22,23]. However, in agreement with our results, other studies reported no significant differences among age-groups[13,14,17]. This inconsistency among results can be explained by the multifactorial nature of the mini-implant success rate. Moreover, it can be argued that Lee *et al*[15] and Chen *et al*[20] have overlooked the evaluation of the effect of gingival tissue type at the placement site.

In our study, the gingival tissue type at the placement site was the main factor affecting the success rate. In AG, the placement of mini-implant had a 2.7 times lower failure rate than in MGJ which in turn had a 1.6 times lower failure rate than in MM (Table 1). This was in accord with previous investigations[5,12,16]. Moreover, an animal study showed a significantly higher stability of mini-implants in keratinized gingiva. Within their limited sample size (22 mini-implants), all failed cases ($n = 9$) were placed in the non-keratinized gingiva[24]. The lower failure rate in the AG could be explained by the non-movable keratinized

Table 1 Mini-implants' success and failure rates, and ORs statistics by host variables *n* (%)

Variables	Success	Failure	Total	*P*-value	OR (95%CI)	
Gender				0.231		
Male	96 (84.96)	17 (15.04)	113			
Female	252 (89.36)	30 (10.64)	282		0.762 (0.354, 1.274)	
Jaw				0.019		
Maxilla	199 (91.71)	18 (8.29)	217			
Mandible	149 (83.71)	29 (16.29)	178		2.152 (1.151, 4.021)	
R/L side				0.877		
Left	117 (88.50)	23 (11.50)	200			
Right	171 (87.69)	24 (12.31)	195		1.080 (0.587, 1.986)	
Age				0.973		
< 20	106 (87.60)	15 (12.40)	121			
20-30	204 (88.31)	27 (11.69)	231		0.935 (0.477, 1.834)	
> 30	38 (88.37)	5 (11.63)	43		0.930 (0.316, 2.732)	
Gingival tissue				< 0.001		
AG	165 (94.29)	10 (5.71)	175			0.367 (0.163, 3.204)
MGJ	109 (85.83)	18 (14.17)	127		2.724 (1.212, 6.124)	
MM	73 (79.35)	19 (20.65)	92		4.294 (1.903, 9.689)	1.576 (0.775, 3.204)

Fisher's exact test. R/L: Right/left; AG: Attached gingiva; MGJ: Mucogingival junction; MM: Mucous membrane.

Table 2 Distribution of mini-implants according to gingival tissue type at the placement site in maxilla and mandible *n* (%)

	Mandible		Maxilla		*P*-value
	Total	Failed	Total	Failed	
Attached gingiva	34 (19.1)	4 (11.8)	141 (65.3)	6 (4.3)	
Mucogingival junction	78 (43.8)	11 (14.1)	49 (22.7)	7 (14.3)	< 0.001
Mucous membrane	66 (37.1)	14 (21.2)	26 (12.0)	5 (19.2)	

Table 3 Independent comparison of failure rate between maxilla and mandible according to gingival tissue type *n* (%)

Variables	Success	Failure	Total	*P*-value	OR (95%CI)
Attached gingiva				0.105	
Maxilla	135 (95.74)	6 (4.26)	141		
Mandible	30 (88.24)	4 (11.76)	34		3.000 (0.797, 11.293)
Mucogingival junction				1	
Maxilla	42 (85.71)	7 (14.29)	49		
Mandible	67 (85.90)	11 (14.10)	78		0.985 (0.354, 2.740)
Mucous membrane				1	
Maxilla	21 (80.77)	5 (19.23)	26		
Mandible	52 (78.79)	14 (21.21)	66		1.131 (0.362, 3.535)

tissue that decreases the susceptibility to irritation and infection. On the other hand, some authors reported no significant differences in the success rate according to soft tissue[14,17,25].

Several studies reported higher success rate of mini-implant placement in the maxilla than that for those placed in the mandible[12,16,20]. On the other hand, some authors reported no significant differences between the upper and lower jaws in mini-implant success rate[11,13,14,25]. In our study, the jaw, initially, was a significant factor affecting the success rate. However, with further analysis, a significant association ($P < 0.001$) was found between the jaw and the gingival tissue type. The mini-implants placed in the mandible were mainly placed in mucous membrane or MGJ, while those placed in the maxilla were mainly in the attached gingiva. No significant differences in the success rates were found between the mini-implants placed in upper and lower jaws when compared within each gingival tissue type.

This was in agreement with Moon *et al*[13] who placed all the mini-implants in the attached gingiva and showed no significant difference in the success rate between maxilla and mandible. In addition, in our results, Cox proportional hazard model showed no significant effect of the

Table 4 Regression analysis of factors affecting the failure rate of min-implants

	Beta	SE	Wald	*P*-value	Exp (B)	95%CI of Exp (B)
Gender	0.444	0.124	12.744	< 0.001	1.559	1.222-1.989
Jaw	0.107	0.109	0.956	0.358	1.113	0.898-1.378
R/L Side	-0.031	0.107	0.085	0.771	0.969	0.785-1.196
Age	-0.027	0.084	0.101	0.751	0.974	0.826-1.148
Gingival tissue	0.162	0.069	5.512	0.012	1.176	1.027-1.347

Cox proportional hazard model. Wald: Wald statistic; Exp (B): Exponential of Beta; R/L: Right/left.

jaw on the failure rate ($P = 0.358$). Therefore, the greater failure rate of mini-implants placed in the mandible can be explained by the lake of further analysis to examine any association between the jaw and other factors, such as inflammation, root proximity, and soft tissue mobility.

Similarly, gender was described as a significant factor in several studies. Moon *et al*[13] reported a higher success rate in male patients while in Antoszewska *et al*[5] study female subjects had a higher rate. Nevertheless, our results showed no significant difference in the rate according to gender. This was in accordance with several reports[14,17,20]. Interestingly, the Cox proportional hazard model in our study showed that gender was a significant factor. Therefore, future studies might be required to evaluate the influence of gender on the success rate with a larger sample size from both groups with uniform inclusion criteria that eliminate other confounding factors.

Time of loading has been evaluated in several reports but no consensus was reached. Trisi *et al*[26] demonstrated that immediate loading might undermine the stability of dental implants and increase the number of failures. On the contrary, other studies showed a positive influence for the immediate loading[12,27]. However, Miyawaki *et al*[11] found no correlation between the time of loading and success rate. In addition, Cheng *et al*[16] and Costa *et al*[28] achieved success rates of 89% and 87.5% with delayed and immediate loading, respectively. In our study, to minimize the effect of the loading time, all mini-implants were loaded three weeks after placement.

From our results, it is recommended that clinicians place mini-implant in the attached gingiva as long as possible to improve the success rate. However, further prospective controlled studies are required to evaluate the efficiency of different types of temporary anchorage devices used for various clinical situations.

In summary, with the single type of mini-implants used by the same clinician, survival analysis was performed to evaluate the success rate of mini-implant. The gender and gingival tissue type had significant effects on the success rate. Mini-implants placed in the attached gingiva had a higher success rate than that of those placed in the mucogingival junction and mucous membrane. However, no significant differences in the success rate were found according to age, gender, and implantation side and jaw. Therefore, it is recommended for clinician to consider the characteristics of gingival tissue prior to mini-implant insertion.

COMMENTS

Background

Anchorage reinforcement is a critical factor for successful orthodontic treatment outcome. Mini-implants are applied to achieve various dental movements such as anterior retraction, molar protraction and distalization, intrusion, extrusion, and correction of midline and occlusal canting.

Research frontiers

With improvement of mini-implant materials, design and placement technique, recent studies have often reported mini-implant success rates higher than 90%. On the other hand, since it is rare that a patient receives only one mini-implant during orthodontic treatment, the success rate faced by clinicians throughout treatment may be substantially lower due to presence of multiple mini-implants in each patient.

Innovations and breakthroughs

With the single type of mini-implants used by the same clinician, survival analysis was performed to evaluate the success rate of mini-implant. The gender and gingival tissue type had significant effects on the success rate. Mini-implants placed in the attached gingiva had a higher success rate than that of those placed in the mucogingival junction and mucous membrane.

Applications

From their results, it is recommended that clinicians place mini-implant in the attached gingiva as long as possible to improve the success rate. However, further prospective controlled studies are required to evaluate the efficiency of different types of temporary anchorage devices used for various clinical situations.

Peer review

This is a well-written and interesting article about mini-implants success rate. English grammar and language are good. The title reflects the major topic and contents of the study. The abstract clearly describes the research background, objectives, materials and methods, results and conclusions. The study design is appropriate, as well as the used statistical methods. The sample size and the statistical data are adequate for this clinical study.

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BRIEF ARTICLE

Management of missile injuries to the maxillofacial region: A case series

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Abstract

AIM: To assess our management of patients suffering from missile injuries to the maxillofacial region.

METHODS: From December 2009 to September 2012, 40 patients with missile injuries (high velocity gunshot and bullet wounds, explosive injuries and shrapnel *etc.*) affecting the maxillofacial region were treated. All except for 2 patients were males. All had soft tissue injuries with or without bone injuries. These patients were referred to the plastic and maxillofacial surgery ward of our hospital. The patients were 19 to 65 years of age (mean 45 years). In 19 cases, there were missile injuries to other parts of the body, especially the lower extremities. All of the patients were managed by early soft tissue debridement, comprehensive reconstruction and antibiotics. This retrospective study was approved by the IRB and ethical committees.

RESULTS: The majority of injuries were caused by high velocity projectiles (88%) and the remaining by car explosions or dynamite blasts (12%). 40 patients were treated surgically. Thirty patients had soft tissue loss (75%) and 20 patients (50%) had bone loss; there was combined soft tissue and bone loss in 10 (25%) patients. Facial fractures were in the orbital bones in 10 cases, maxillary in 7, nasal in 5 and the mandible in 3 cases. We used primary repair in the majority of soft tissue defects (25 of 40 cases). Bone repair was done primarily at the same stage using miniplates, titanium screws or wires. In some cases with a bone defect, iliac bone grafts were used simultaneously or in the later stages (mandibular defects). There was no failure of bone reconstruction in our cases. Infections occurred in two cases and were treated with systemic antibiotics and dressing changes, without any long term sequelae.

CONCLUSION: Our principles for soft tissue reconstructions were according to the reconstructive ladder and included primary repair, local flaps, skin grafts and regional flaps depending on the extent of damage. Primary repair in facial missile defects was not associated with increased morbidity or complications in this series. We recommend this approach when feasible.

Key words: Missile; Maxillofacial; Management; Primary; Surgery

Core tip: Exposure to missile injuries may result in unique and complex injury patterns from projectiles or fragments. Injuries to the face due to firearms are either high velocity or low energy; high velocity projectiles can result in devastating functional and aesthetic consequences, shattering the hard tissues. Early intervention in facial firearm injuries resulted in restoration of occlusion and continuity of the jaw, fixation of luxated or extruded teeth, early return of function, prevention of segment displacement and tissue contracture,

less scarring and decreased need for major bone graft reconstruction later on.

Ebrahimi A, Motamedi MHK, Nejadsarvari N, Kazemi HM. Management of missile injuries to the maxillofacial region: A case series. *World J Stomatol* 2013; 2(3): 62-66 Available from: URL: http://www.wjgnet.com/2218-6263/full/v2/i3/62.htm DOI: http://dx.doi.org/10.5321/wjs.v2.i3.62

INTRODUCTION

Missile injuries to the maxillofacial region are important health issues, both in the military and civilian population. The range of damage of these injuries represents a continuum of severity from minor injuries to those resulting in lost workdays, long-term disability and fatalities [1].

We managed such patients after primary urgent management at our hospital. The principles of treating blunt trauma to the face are well established; however, missile injuries in this region have special features that provide the surgeon with multiple medical and surgical challenges when dealing with these injuries[2].

Severity

The severity of these injuries depends upon many factors, including the type of missile and type and site of the injury; damage to the tissue is much more a function of the velocity of the missile than of its mass[2].

Assessment and resuscitation

The most important factor in the care of patients with a missile injury is the initial assessment and resuscitation performed at the emergency department.

Management

The management of missile injuries of the maxillofacial region can be divided into three phases: immediate, intermediate and late[3]. Indeed, most plastic and maxillofacial surgeons manage patients in the intermediate and late phases but require cooperation between the emergency physician and maxillofacial surgeon for optimal and early management.

Controversy exists regarding early aggressive intervention or a more conservative approach[2]. In this article, we review facial reconstruction after missile injuries with early surgical intervention.

MATERIALS AND METHODS

Forty patients with missile injuries (high velocity gunshot wounds, explosive injuries) affecting the maxillofacial region were included in this retrospective study within the period from December 2009 to September 2012 consecutively. These patients were referred to the plastic and maxillofacial surgery ward of our hospital; 38 patients were men and 2 were women, with an age range of 19 to 65 years (mean 45 years). All patients had combined soft tissue with or without bone injuries in the facial region (Figure 1). In 19 cases, there were missile injuries to other parts of the body, especially the lower extremities. We managed all patients with early soft tissue debridement and reconstruction and placed them on antibiotics for one week after primary surgery. This study was approved by the IRB and ethical committee.

Soft tissue management

Our principles of reconstruction of the facial soft tissues were by primary intention, including primary repair, local flaps and regional flaps such as cervicofacial flaps. In periorbital wounds, the orbit and globe were examined carefully for detection of injuries and we requested an ophthalmologist consultation for such patients (Figures 2 and 3). In three cases of gunshot injury with unilateral blindness, globe enucleation was done.

Shell fragments

Shell fragments, bullets and shrapnel were removed if they were in the field of operation; otherwise they were left.

Bone management

For bone reconstruction, we restored shape, contour rigidity and stability to the facial skeleton with different devices, such as titanium screw and plate, wire and arch bar immobilization with or without bone grafts (Figure 1). Our method for bone grafting was in the early phase from the iliac crest.

Mandible

For treatment of gunshot wounds of the mandible, we used an intraoral or extraoral approach and open reduction and internal fixation with miniplates or reconstruction plates, with or without intermaxillary fixation. In one case, we used a reconstruction plate (Figure 1) and in a later stage iliac bone grafting was done.

Maxilla

For treatment of gunshot wounds of the maxilla, we used intraoral incision and open reduction and internal fixation with miniplates with or without intermaxillary fixation.

Frontal bone

For treatment of gunshot wounds of the frontal bone, we used an open approach from the laceration site or coronal incision and, after reduction, internal fixation with miniplates with or without bone graft was done.

Periorbital fractures

All periorbital fractures were operated on during the first week after injury. Upon admission of injured patients, examinations were done as indicated (radiography, axial and coronal facial CT scans, Doppler ultrasound for carotid artery damage).

Orbital fractures

For treatment of gunshot wounds of the orbit, we used

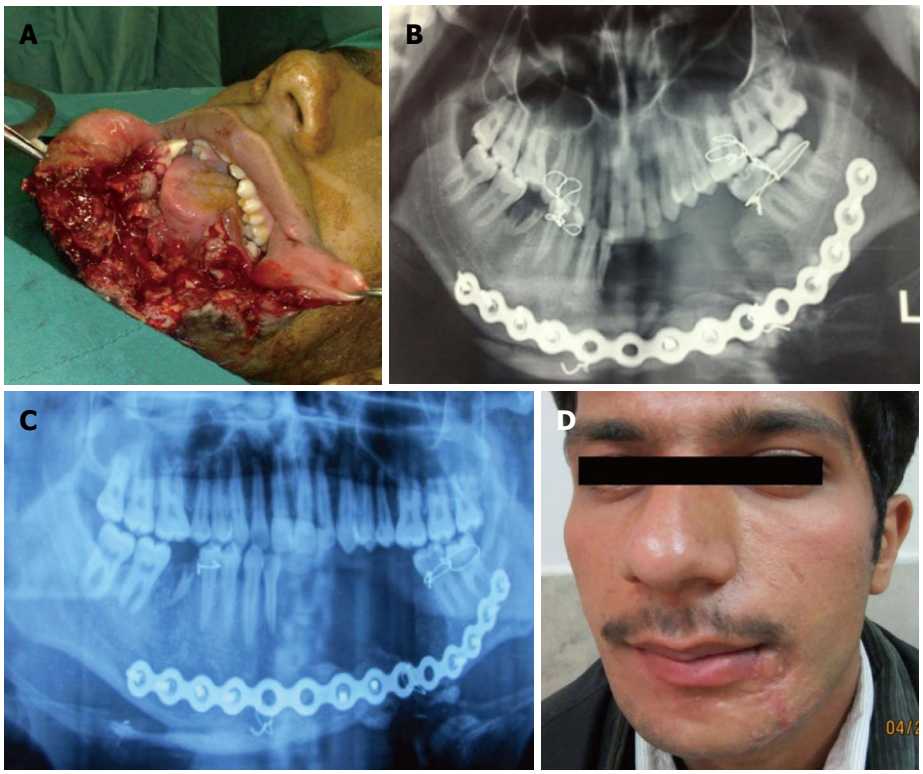

Figure 1 A 20-year old man with a gunshot wound to the lower face, with disruption of soft tissue and the mandible bone in body with bone defect. A: Before operation; B: Computed tomography scan before operation; C: Reconstruction with reconstructive plate; D: Three months post operation.

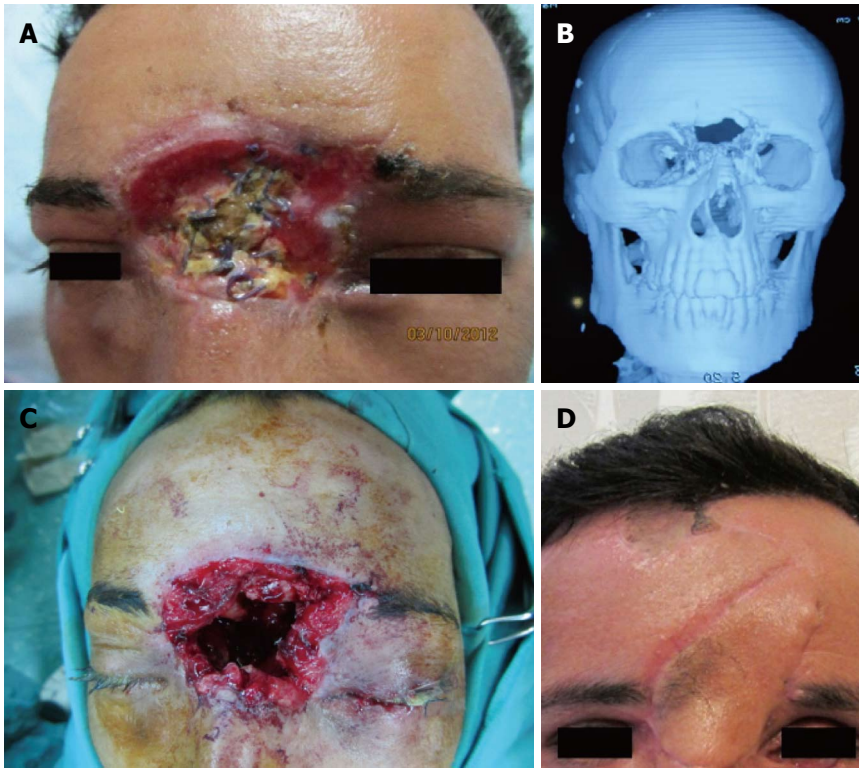

Figure 2 A 30-year old man with a gunshot wound to the upper face, with disruption of the forehead and frontal and ethmoid sinus and left eye to the base of the skull. A: Before operation; B: The patient was treated with abdominal fat to obliterate the frontal sinus elsewhere before referral. Computed tomography scan before reconstruction; C: Intra operative view; D: Reconstruction with forehead flap and iliac bone graft (one month post operation).

an open approach with reduction and internal fixation with miniplates and screws, with or without an implant.

Scars

All deformities and scar contractures were corrected after maturation of scars. Ophthalmic injuries were diagnosed in 10 patients (globe, eyelid and eyebrow). Enucleation of the unilateral eye was done in 3 cases by the ophthalmologist for severely damaged and complete blindness of the unilateral eye.

We used primary repair in the majority of soft tissue defects (25 of 40 cases). Bone repair was done primarily at the same stage using miniplates, titanium screws or wires. In some cases with a bone defect, iliac bone grafts were used

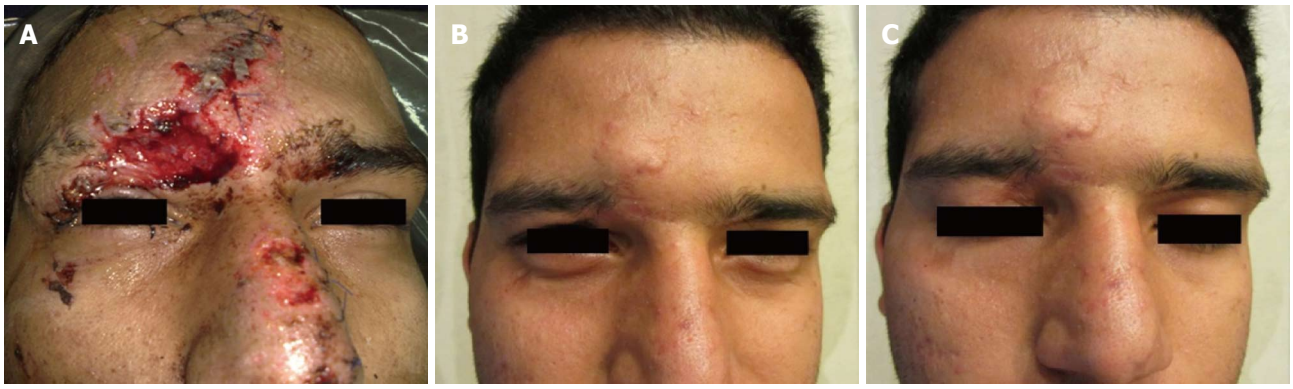

Figure 3 A 23-year old man with a gunshot wound defect of the eyebrow. A: Before operation; B: Early post operation; C: One year post operation.

Table 1 Distribution of facial soft tissue and bone damage in 40 gunshots and blast injured persons

	Site of injury	*n*	Bone	Soft tissue defect	Combined soft tissue-bone
1	Periorbital	12	10	6	4
2	Maxillary	17	7	16	6
3	Mandible	4	3	2	1
4	Nasal	5	3	4	3
5	Frontal	2	2	2	2
Total		40	25	30	16

simultaneously or in the later stages (mandibular defects).

RESULTS

A total of 40 patients were treated and followed from 5 months to 3 years. There were 38 male and 2 female patients, with an average age of 37 years (range 19-65 years). Most injuries were caused by high velocity projectiles (88%) and the remaining by car explosions or dynamite (12%). Thirty patients had soft tissue loss (75%) and 20 patients (50%) had bone loss; there was combined soft tissue and bone loss in 10 (25%) patients. Facial fractures were in the orbital bones in 10 cases, maxillary in 7, nasal in 5 and the mandible in 3 cases (Table 1).

There was no failure of bone reconstruction in our cases. Infections occurred in 2 cases and were treated with systemic antibiotics and dressing changes, without any long term sequelae.

DISCUSSION

Exposure to missile injuries may result in a unique and complex injury pattern, usually from fragments or bullet wounds which are often fatal if they involve the head. Blast overpressure is the abrupt, rapid rise in atmospheric pressure resulting from explosive detonation, firing of large caliber weapons and accident occupational explosions[4,5]. There are two schools of thought for the management of such patients subjected to missile injuries: early intervention and nonaggressive conservative intervention[2]. Injuries to the face due to firearms are either high velocity or low energy; high velocity projectiles can result in devastating functional and aesthetic consequences, shattering the hard tissues[6].

Our principles for soft tissue reconstructions were according to the reconstructive ladder, including primary repair, local flaps, skin grafts and regional flaps depending on the extent of damage (Figure 3). We used primary repair in the majority of soft tissue defects (25 of 30 cases) and recommend this approach for these injuries.

We used surgical intervention in all cases. Early intervention in facial firearm injuries resulted in restoration of occlusion and continuity of the jaw, fixation of luxated or extruded teeth, early return of function, prevention of segment displacement and tissue contracture, less scarring and decreased need for major bone graft reconstruction later in one study[6]. If continuity of the mandible can be obtained, in the subsequent operations there will be no need for maxillomandibular fixation. In this case series study, we had no major complications after early surgical interventions.

All facial wounds were under systemic antibiotic therapy for one week and local antibiotic ointment to prevent secondary infections. There was no failure of bone reconstruction; in our cases, maxillary defects were reconstructed with bicortical bone grafts in the same operation. We had facial wound infections postoperatively in 2 cases and treated them with systemic antibiotics and dressing changes, without any long term sequelae (these two patients had a mandible fracture with a through wound of the oral cavity without any medical immunocompromising factors).

The issue of when to treat maxillofacial firearm injuries remains controversial (early or delayed), although not all maxillofacial projectile injuries can be comprehensively treated at the onset[7]. Although all missile wounds are contaminated, the general consensus in the medical literature and textbooks consider these infections to be mostly of odontogenic origin[8]. In composite defects (soft tissue and bone), we used bone graft and soft tissue flaps simultaneously for coverage of bone and our results were free of any significant resorption or flap necrosis after early operative intervention.

In our study, the most common site of entrance and exit wounds was in the cheek (67%). In another study in Iraq by Kummona, the most common site was also in the cheek

Table 2 Associated injures in the face *n* (%)

Associated injures	
Facial nerve	2 (5)
Parotid duct	4 (10)
Globe	3 (7.5)
Oral mucosa	3 (7.5)
Lacrimal duct	2 (5)
Total	14 (35)

(54.8%). According to our results, the midface is a common site for gunshot injuries and a safe coverage for protection of the cheek in military and civilian people must be designed for combat. The face is the part of the body most subjected to injuries, either by road traffic accidents or missile war injuries[9]. In our experience, gunshot injuries of the craniofacial region are not a single site injury and often have associated injuries; thus, a complete evaluation of soft tissue and bones must be done for all patients. We used free bone grafting for 4 patients in our cases and the preferred site for bone graft harvesting was the iliac crest because of combined cortico-cancellous block of bone. For delayed reconstruction of the frontal cranium, we used titanium mesh and soft tissue flap with acceptable results (Figure 3) and without any complications. In our series, the most common associated injuries were ophthalmic injuries (Table 2), seen in 10 patients with unilateral blindness. In another study, the most common injured facial structure was the facial nerve and the second most common was ophthalmic injuries. An important problem in patients with gunshot injuries or blast damage is facial burn blast tattoos that must be managed early post damage by operative intervention. Application of silver sulfadiazine before the operative intervention helps to remove embedded particles better and decrease traumatic tattoos[10]. This procedure is also better to be done early. Advocates of primary management have supported this viewpoint[11-14].

In high velocity gunshot and blast injuries with facial damage to soft tissue and bone, early surgical intervention is beneficial and good results without significant complications can be obtained; we recommend this approach in these types of facial injuries.

COMMENTS

Background

Firearm projectiles can result in devastating functional and aesthetic consequences, shattering the hard tissues. Early intervention in facial firearm injuries is still in debate.

Research frontiers

Experience has shown that early management results in restoration of occlusion and continuity of the jaw, fixation of luxated or extruded teeth, early return of function, prevention of segment displacement and tissue contracture, less scarring and decreased need for major bone graft reconstruction later on.

Innovations and breakthroughs

Innovations in surgical instruments and hardware, plates and screws have enabled rigid fixation of fractures.

Applications

These advancements have enabled surgeons to better manage gunshot injuries.

Peer review

The manuscript is a result of good efforts on the part of the authors and may be useful to determine management of maxillofacial injuries secondary to missiles and gunshot wounds.

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EDITORIAL

Pharmacogenomics in oral diseases

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Abstract

The availability of newer technologies for identification and characterization of the human genome has enabled our understanding of the genetic variations in a majority of human diseases. Human genomic sequence varies in less than 1% among the different population group and these differences known as gene polymorphisms are the primary reasons for differences in individuals' response to various drug therapy. Also understanding the genetic changes may enable implementation of targeted therapy, thus providing for effective treatment strategies and minimizing the adverse side effects. Pharmacogenomics is a recent development in the field of personalized medicine which focuses on the genetic determinants of drug response at the levels of entire human genome. It primarily deals with tailoring of drug therapy for every individual based on their genetic make-up and identifying new target in various diseases for drug therapy. While the application of pharmacogenomics in systemic illness is well researched, its role in oral diseases needs documentation. Identifying specific targets in periodontitis, head and neck cancer, infections and genetic disorders can be beneficial in discovery of new drugs. This editorial provides an overview of basics of pharmacogenomics, its current role in disease management and its potential role in various head and neck diseases.

Key words: Pharmacogenomics; Oral Cancer; Periodontal diseases; Genomic variations; Targeted drug therapy

Core tip: Pharmacogenomics mainly focuses on the genetic determinants of drug response at the level of entire human genome. It primarily deals with tailoring of drug therapy among every individual based on their genetic make-up and identifying new targets in various diseases for drug therapy. Identification of gene polymorphisms in humans will aid in modulating drug therapy for individual needs as well as leading to discovery of target drugs. This editorial provides an overview of basic pharmacogenomics and its usefulness in oral diseases.

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INTRODUCTION

Pharmacogenomics is a component of individualized medicine focusing on how genetic factors influence individual responses to different medications that may affect drug efficacy, side effects and adverse events to drug therapy[1]. The aim of pharmacogenomics is to decrease adverse responses to therapy through determining new therapeutic targets and genetic polymorphisms that affect drug specificity and toxicity[2]. The term pharmacogenomics, a relatively new term is often used interchangeably with a much older term pharmacogenetics though differences exist between the two terms. While pharmacogenetics refers to the study of how individual genes in-

fluences the response to medications, pharmacogenomics is related to the study of how individuals' genomic composition as a whole affects their response to medicine[3]. The field of pharmacogenomics uses genetic and genomic information of individuals in order to predict the response of patient groups to drugs and thus guide clinical trial and the drug development process. This can be made possible with the development of human genome project which encodes majority of human genes.

Most of the commonly occurring diseases such as cancer, atherosclerosis and neurodegenerative disorders comprise of a group of genetically discrete entities with separate molecular etiologies and possibly different responses to therapy. Pharmacogenomic therapy has been attempted to treat cystic fibrosis[4], acquired immune deficiency syndrome (AIDS), cardiovascular diseases, psychiatric illness and targeted therapy towards epidermal growth factor receptor (EGFR) for treatment of pancreatic cancer[5] and non small cell lung carcinoma[6]. In addition to the development of new therapeutic agents, pharmacogenomics can also predict outcomes and beneficial therapy regimens. The various targets that are extensively studied are angiotensin converting enzyme regulating cardiovascular functions; analyzing the association between β2 adrenergic receptor polymorphism and asthma[7] and use of apolipoprotein polymorphisms to predict the risk of heart disease and response to treatment[2].

Most of the disorders affecting the head and neck region excluding trauma have genetic implications in various forms. The disorders range from developmental disturbances, microbial infections and bone disorders to various forms of head and neck cancers. An understanding of the genetic changes occurring in these disorders may be helpful in implementation of drug therapy aiming to obtain maximum efficacy with minimum side effects and also to evolve targeted drug therapy for the management of such lesions.

BASICS OF PHARMACOGENOMICS

A gene is defined as a specific sequence of nucleotide bases whose sequences carry the instructions for the constructions of proteins. Hundreds of genes reside on each chromosome and the complete human genome is estimated to contain about 30000 genes[8]. More than 99% of DNA sequence is the same across the entire human population. The small genetic dissimilarities have a major impact on persons' physical makeup and response to disease as well the effectiveness of the therapy instituted. Pharmacogenomic technologies try to detect these genetic variations in a patient or patient populations to help select drug compounds and doses that are more likely to work.

These genetic variations which account for around 1 million of the 3 billion bases of human genome are termed as polymorphisms. Polymorphisms arise from three fundamental types of DNA sequence variations namely single nucleotide polymorphisms (SNPs) representing nucleotide substitutions, insertions or deletions and indel of repetitive DNA[9]. Advances in pharmacogenomics mainly depend on identification of SNPs in the human genome. These arise from mutations affecting a single nucleotide, occurring relatively frequently and must exceed in a population of a frequency of 1% to meet the requirement of genetic polymorphism[10]. The main research use of a human SNP map would be to determine the contributions of genes to diseases that have a complex multifactorial basis. More than 1.4 million SNPs have been identified in human genome till date[8]. Majority of the genetic polymorphisms are found in drug metabolizing enzymes, receptors and transport proteins and produce varying effects on drug metabolism[2]. The use of these genetic variations in order to individualize drug therapy and to identify novel targets to enable the development of new drugs for various diseases are the primary reasons for which pharmacogenomics is employed.

Drugs in the body are metabolized by enzymes and most of the enzymes belong to the members of cytochrome P450 (CYP) system. These enzymes are located in the liver and gastrointestinal tract and include greater than 30 isoforms[11]. Individual variations in the genes that produce these enzymes causes different people to metabolize the same drug differently; less active or inactive forms of CYP enzymes that are unable to breakdown and efficiently eliminate a drug from the body (slow metabolizers) can cause the drug to build up while very active forms (rapid metabolizers) can cause the body to clear itself of a drug before it has a chance to work[12]. Understanding an individuals' response to a certain drug can help the clinician to decide the accurate drug dosage required for effective therapy thereby reducing the chances of overdose or insufficient dosage. Of all the types of CYPs, most of the functional genetic polymorphisms reside in only few of them namely CYP3A4, CYP2A6, CYP2C9, CYP2C19 and CYP2D6[10]. Of these, CYP3A is involved in the oxidative biotransformation of up to 50% of clinically important therapeutic agents that has resulted in the withdrawal of important drugs like Mibefradil (anti-hypertensive drug), Rezulin (oral anti-hyperglycemic agent) and propulsid (for treatment of gastrointestinal disorders).

Other less common polymorphisms can be seen in drug transport proteins and receptors. Transport proteins are proteins that allow compounds to be transported across cell membranes and P-glycoprotein is a drug transport protein known to be involved in the metabolism of many drugs[13]. Identifying this polymorphism would be a valuable tool in determining therapeutic concentrations required for individual patients. Receptors polymorphisms are helpful for development of new therapeutic agent and to predict outcomes and beneficial treatment regimens. Some of the common receptor targets that have been targeted by the use of drugs include angiotensin converting enzyme, β2-adrenergic receptor polymorphism, cystic fibrosis transmembrane conductance regulator and p53 and EGFR for anti-cancer therapy.

APPLICATIONS OF PHARMACOGENOMICS IN ORAL DISEASES

Genetic mutations are a common finding in majority of the human diseases affecting the head and neck region. which may contain one or many genetic polymorphisms. Examples of disorders exhibiting single gene mutations include Treacher-Collins syndrome, Pierr-Robin syndrome, Crouzon syndrome, Ectodermal dysplasia, achondroplasia and Gorlin syndrome while cleft lip and palate, congenitally missing teeth, dental caries, severe malocclusion, head and neck cancer, periodontal diseases and autoimmune disorders are caused by multiple gene mutations[14].

Chemotherapeutic intervention for cancer therapy is undergoing changes from being an empiric random screening approach to a target directed approach where specific abnormalities in cell functioning are modulated in a drug receptor fashion. The use of small molecules with tyrosine kinase inhibitory activity directed towards the EGFR such as gefitinib and erlotinib are used for treatment of NSCLC, pancreatic and breast cancer[5]. EGFR is a transmembrane glycoprotein member of erbB family of type I tyrosine kinase which plays a crucial role through downstream signaling pathways in cell cycle progression, survival and proliferation[15]. Overexpression of EGFR in head and neck cancer are known to be associated with poor prognosis and hence the use of drugs such as tyrosine kinase inhibitors may help in improving the prognosis and survival rate. Adequate understanding of the molecular mechanisms involving various growth factors (such as transforming growth factor, platelet derived growth factor, hepatocyte growth factor), cytokines and genetic mutations occurring in carcinogenesis will aid in the development of chemotherapeutic drugs against specific targets for appropriate management and to reduce patient morbidity and mortality.

Periodontitis is a polymicrobial infection resulting from a complex interaction between oral microbes and host immune response leading to periodontal destruction and alveolar bone resorption. The host response to infection is primarily in the form of inflammatory reaction leading to release of various cytokines, growth factors and matrix metalloproteinases (MMP). Identification of therapeutic targets which are directed towards the specific host alteration may be helpful as an adjuvant treatment for periodontitis. Monoclonal antibody derivatives directed towards MMP are promising as therapeutic agents and mainly involves non-antimicrobial activities of low dose tetracycline and tetracycline analogue doxycycline hyclate via the inhibition of MMP-8 and -13 protease mechanisms. The therapeutic action of these agents is primarily due to the modulation of the host response because the low dose formulations of these drugs have lost their antimicrobial property[16]. Research in targeted therapy are also underway for treatment of various other microbial infections like candidiasis, birth defects, orthodontic tooth movements but are still at initial stages.

To summarize, decoding the human genome with aim to describe genetic changes in various oral diseases will be beneficial in providing appropriate therapy. The use of pharmacogenomics in determining the functioning of various drugs in individual patients will be a boon for clinicians to decide the appropriate dosage. Research concerning targeted drug therapy has advanced exponentially and could be ideal for treatment of diseases like cancer and AIDS without major side effects and also for management. This has been enhanced by the availability of advanced technologies such as SNPs and DNA microarrays which helps in analyzing genetic changes.

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Stomatological management of head and neck cancer patients treated with chemotherapy and radiotherapy

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Abstract

Treatment of head and neck cancer with radiotherapy and/or chemotherapy can cause oral damage. Long-term treatment can damage the salivary glands, the oral mucosa, and the maxilla, leading to altered production of saliva and to multiple infections. These lesions can be prevented, limited or avoided by thorough evaluation prior to treatment and by therapeutic follow-up and preventive measures. The dentist must have strong medical knowledge of the possible short-, medium-, and long-term oral complications of the cancer treatment, and must have knowledge of the protocols for oral management of cancer patients. The availability of a multidisciplinary medical team together with a dentist to attend to the patient prior to the cancer treatment, as well as close communication between team members during and after treatment, is crucial. The aim of the present study was review the stomatological management of head and neck cancer patients treated with chemotherapy and radiotherapy and summarizing current treatments, therapeutic innovation and tissue regeneration perspectives.

Key words: Stomatological management; Head and neck; Cancer

Core tip: The aim of the present study was to conduct a review of therapeutic advances in the prevention and management of oral disorders in head and neck cancer patients receiving radio- and chemotherapy. The study focuses on possible risk factors and on the prevention of these disorders.

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INTRODUCTION

In recent decades, an increase in the prevalence of oral cancer has been observed in several countries. Surgery, radiotherapy and chemotherapy continue to be the treatments of choice for such cancers, and advances have been made in minimizing their adverse effects[1]. However, treatment of cancer with radiotherapy and/or chemotherapy can cause oral damage. Long-term treatment can damage the salivary glands, the oral mucosa, and the maxilla, leading to altered production of saliva and to multiple infections[2]. The surgical treatment of oral and maxillofacial neoplasms can lead to sequelae such as limited speech, eating disorders, alterations in the patient's sense of taste and smell, and changes in the patient's physical appearance[3,4]

Lesions of the oral cavity secondary to head and neck

cancer treatment can be prevented, limited or avoided by thorough evaluation prior to treatment and by therapeutic follow-up and preventive measures[5].

The dentist must have strong medical knowledge and be continuously updated on common head and neck malignant neoplasms, their clinical manifestations, therapeutic alternatives, and the complications that may occur as a result of their treatment[6].

The availability of a multidisciplinary medical team consisting of an oncologist, a hematologist, a head and neck surgeon, a radiologist, a physiotherapist, a speech therapist, a social worker, and a psychologist together with a dentist to attend to the patient prior to the cancer treatment, as well as close communication between team members during and after treatment, is crucial[5,7].

Ideally, cancer centers should provide oral health care. However, because patients are frequently referred to the family dentist, dentists must have basic knowledge of the protocols for oral management of cancer patients and of the relevant National Cancer Institute guidelines[8].

INITIAL CONTACT WITH THE PATIENT

It is important for the dentist to join the oncology team so that he or she is informed of the type of surgery and radio- and/or chemotherapy the patient will receive[5,9].

At the patient's first visit, the dentist must perform a complete oral health evaluation to establish an integral oral and maxillofacial management plan before the cancer treatment is initiated[5].

The possible short-, medium- and long-term oral complications of the cancer treatment must be explained to the patient and to his or her close relatives[10] in a simple and didactical way, preferably through the use of images, so that they will be able to identify problems such as xerostomia and mucositis. Patients must be instructed in the importance of dental follow-up care before, during, and after chemo- and radiotherapy as a means of preventing radiation-associated dental caries and osteonecrosis. The dentist must provide the patient and the patient's relatives with an instructional manual on oral hygiene, diet, and measures to be followed before, during, and after cancer treatment[11-14].

The primary objective of providing information to the patient and of diagnosing and treating the patient prior to cancer treatment is to eliminate or stabilize any oral lesions that are present and to minimize the possibility of the occurrence of local or systemic infections during or after cancer treatment[14-16].

Patients often show tumor-related symptoms or dental conditions related to an incisional biopsy or pre-surgical therapy. These symptoms must be evaluated and correctly diagnosed so as to differentiate tumor symptoms from previous oral manifestations of dental caries, periodontal disease, pulpal diseases and soft tissue conditions[13,17].

A detailed exploration of the head and neck region must be performed following a preset order from external to internal, and abnormal growth, asymmetries, and cutaneous lesions must be identified and evaluated. Salivary glands, muscles, and the temporomandibular joint must be inspected. Palpation of submental, submandibular, and cervical lymph nodes is important and must be followed by an intraoral examination starting with the soft tissues, buccal mucosa, tongue, floor of the mouth, and the hard and soft palates. Any lesion, irritation, erosion, ulceration, or hemorrhage must be identified, and the patient's general periodontal state must be evaluated as well[18].

It is essential to note the presence of caries, damaged restorations, pulpal lesions, necrotic teeth, or apical lesions suggestive of a cyst or granuloma.

The clinical diagnosis is complemented by X-ray imaging with a full set of periapical radiographs and by orthopantomography.

PREVIOUS DENTAL TREATMENT

The essential steps of dental pre-treatment prior to anticancer therapy are focused on the elimination or stabilization of oral lesions and are aimed at minimizing the presence of potential sites of infection during or after treatment[19,20].

The important goals of such pre-treatment are as follows: (1) To eliminate any deep carious processes that may compromise pulp vitality during cancer treatment; (2) To control pulp and periapical infections two weeks before therapy to ensure tissue healing; (3) To restore or extract any tooth that shows a periapical lesion because such teeth can become infection sites in patients receiving chemo- and radiotherapy and hese treatments affect the immune response; (4) To extract teeth with poor periodontal or pulpal prognosis, such as teeth with deep caries or deep periodontal pockets and non-vital teeth with an expectancy of less than one year in the mouth. The extraction should be performed as soon as possible and at least three weeks before cancer therapy begins to ensure that the healing process is completed before the onset of therapy; (5) To assess the need for extraction of teeth associated with the tumor or radiation site; (6) To eliminate or restore sharp edges of fractured teeth to reduce mucosal friction or trauma that could aggravate mucositis; (7) To assess the need for extraction of retained teeth and impacted third molars that can cause pericoronitis; (8) The use of pit and fissure plaque sealants on recently erupted teeth is recommended; (9) To perform dental hygiene and scaling to completely eliminate dental and supra- and infra gingival tartar; (10) To inform the patient of the need to change a cariogenic diet[7] and to suspend the consumption of alcohol, tobacco, and any foods or substances that can damage oral structures; (11) To evaluate the need for adjustment or removal of partial or complete dentures or orthodontic appliances that can cause irritation or trauma. During the cancer treatment, dentures must be used by the patient only when eating; and (12) To encourage the patient to maintain proper oral hygiene and to emphasize a preventive treatment aiming on remineralization to minimize caries formation. The patient must be advised to: use fluoride toothpaste; brush his/her teeth four times a day, including after every meal;

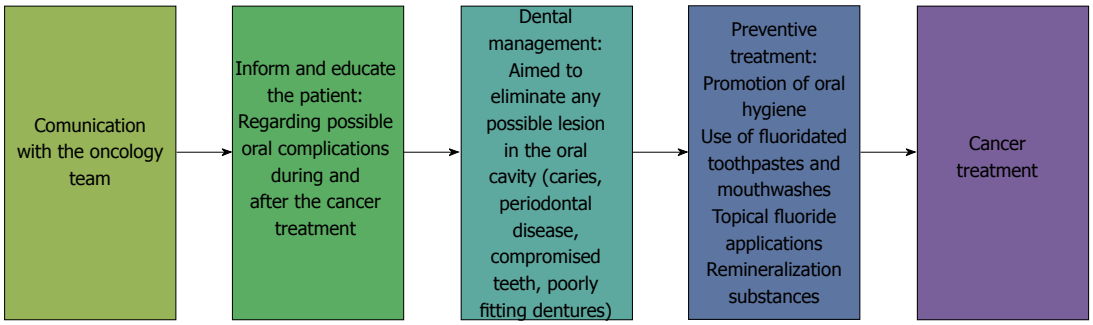

Figure 1 Steps the dentist should follow before cancer treatment is initiated.

use topical fluoride gel daily for 5 min at bedtime; use a calcium phosphopeptide remineralization cream; use alcohol-free fluoride mouthwash.

The diagnostic, preventive, and therapeutic steps that should be followed prior to cancer treatment are shown in Figure 1.

PATIENTS UNDERGOING RADIOTHERAPY

Conventional radiotherapy is very useful in the treatment of oral carcinoma; however, it acts on both tumor cells and healthy cells, producing tissue damage. Approximately 50% of malignant head and neck neoplasms are treated with radiotherapy alone or with chemotherapy and surgery. Radiotherapy involves the use of ionizing radiation, which produces morphological and functional changes in tissues and has chemical effects, included the hydrolysis of intracellular constituents and the rupture of DNA strands[20].

The response of tissue to radiation depends on a variety of factors, including the received dose, the fractionation dose, the nature of the radiation, the previous condition of the irradiated tissue, the degree of cell differentiation, cellular kinetics, cell temperature, and the tumor's sensitivity to radiation, location and oxygenation[21].

ORAL COMPLICATIONS OF RADIOTHERAPY

Complications are classified depending on their time of appearance (immediate, medium and late side effects), their intensity, and as reversible or irreversible. Immediate complications appear within 1 wk of treatment and may include erythema, mucositis, dysgeusia, glossodynia, infections (candidiasis, herpes), xerostomia, periodontal disease, severe necrosis, and alopecia. Medium-term complications appear after the third month of treatment and may include trismus, caries, dysphagia, and dental hypersensitivity. Late side effects appear months after the treatment and may include osteoradionecrosis, alterations in tooth development (agenesis, coronal hypocalcification such as enamel hypoplasia, and root alterations such as root shortening, early canal closure, and dilaceration), pulpal necrosis, and pain. Table 1 shows the most frequent complications of radiotherapy classified by time of appearance and prognosis[22].

Preventive measures and management of oral complications of radiotherapy

The occurrence of oral complications of radiotherapy (Figure 2) can be minimized by taking the following preventive actions: Educating the patient about the importance of oral hygiene and emphasizing the cessation of toxic habits such as alcohol and tobacco consumption. Performing professional dental cleaning including tartar removal, root scaling and planing. Eliminating areas of trauma resulting from ill-fitting dentures and sharp edges. Suspending the use of mucosa-supported dentures for 15 d after radiotherapy begins; if possible, suspending their use indefinitely or using them moderately to avoid trauma. Protecting salivary glands and the mucosa of areas that do not require irradiation. Performing quantitative sialometry to evaluate the production of saliva after radiation doses. Extracting compromised or severely damaged teeth (severe periodontal disease, mobility, fractures, caries). Performing conservative dental treatments that include restorations and root canal therapy. Applying topical fluoride before, during, and after radiation treatment. Recommending the use of 0.12% chlorhexidine mouthwashes. Applying pit and fissure sealants to recently erupted premolars and molars in pediatric patients. Modifying the cariogenic diet. The first side effects of a cariogenic diet may become obvious after radiotherapy.

PATIENTS UNDERGOING CHEMOTHERAPY

Chemotherapy in cancer treatment consists of the use of cytotoxic drugs that are intended to destroy or avoid the proliferation of tumor cells. This therapy is not selective; it affects both tumor cells and normal cells, especially cells that undergo rapid cell cycling for continuous replacement. Such cells include bone marrow cells, cells of hair follicles, and gastrointestinal epithelial cells, including oral mucosal cells[23].

Cisplatin, cyclophosphamide, methotrexate, bleomycin, 5-fluorouracil, and vinblastine are used most frequently in the treatment of head and neck neoplasms[24]. The use of

Table 1 Common complications of the head and neck region following radiotherapy

Complication	Characteristics	Time of appearance	Prognosis
Erythema/ radiodermatitis	Redness/decreased skin thickness; skin dryness due to epidermal basal cell damage	Immediate	Reversible
Mucositis	Generalized inflammation of the oral mucosa due to basal cell damage; scaling, mucosal ulcerations	Immediate	Reversible
Dysgeusia	Altered taste (especially to sour and acid tastes) due to taste bud damage	Immediate	Reversible
Glossodynia	Pain and burning sensation in the tongue due to taste bud damage and inflammation	Immediate	Reversible
Candidiasis and Herpes simplex	Secondary infections resulting from loss of mucosal protection caused by mucositis and xerostomia	Immediate	Reversible
Xerostomia	Decrease in salivary flow and dryness of the mouth caused by alterations in salivary glands	Immediate	Irreversible at high radiation doses (more than 60 Gy)
Periodontal disease	Inflammation of the periodontium due to augmented plaque from decreased salivary flow	Immediate	Reversible
Alopecia	Hair loss from hair follicle atrophy	Immediate	Reversible
Severe necrosis	Loss of tissue, scurvy and malodorous ulcerations	Immediate	Irreversible
Trismus	Reduced mouth opening caused by fibrosis of the muscles of mastication or of the temporomandibular joint	Medium-term	Reversible/irreversible
Caries	Damage to the cement-enamel junction, incisal edges and cusps caused by decreased salivation	Medium-term	Irreversible
Dysphagia	Difficulty swallowing food caused by oropharyngeal alterations; may be evidenced by malnutrition	Medium-term	Reversible
Dental hypersensitivity	Dental sensitivity caused by radiation	Medium-term	Reversible
Osteoradionecrosis	Aseptic necrosis of the irradiated bone	Late	Irreversible
Tooth germ alterations	Alteration in odontogenesis in pediatric patients	Late	Irreversible
Pulp necrosis and pain	Pulp necrosis and pain	Late	Irreversible

these drugs may affect the basal epithelial cells that make up the oral mucosal epithelium. When these cells are damaged, the replacement of the epithelium is compromised and scaling and ulcerations of the mucosa occur. Furthermore, xerostomia caused by salivary gland damage may occur, with resulting alterations in the levels of saliva protectors and ageusia. A high percentage of patients present oral infections, bleeding, or a combination of both, and more than 50% of patients also present complications from surgery and head and neck radiotherapy. Most patients who receive high doses of chemotherapy for head and neck cancer develop severe mucositis[24-26].

Bone marrow suppression is one of the most common side effects of chemotherapy. It is often evident in peripheral blood after 10-14 d of treatment; typical signs are leukopenia, neutropenia, thrombocytopenia and anemia. Hair loss, nausea, vomiting, and palmoplantar erythrodysesthesia syndrome are common. The latter is a palmoplantar erythema with erosions, burning sensation, and local pain[27].

PATIENTS UNDERGOING BISPHOSPHONATE TREATMENT

Bisphosphonates are a group of drugs used for the prevention and treatment of bone resorption diseases such as maxillofacial cancer, bone metastasis, malignant hypercalcemia, osteoporosis, Paget's disease, and multiple myeloma. Their structure is based on that of pyrophosphate, a metabolite that regulates the precipitation and extraction of bone minerals; similar to pyrophosphate, they are sensitive to hydrolysis by phosphatases[28-30].

When bisphosphonates are incorporated into bone, osteoclast-mediated bone resorption is prevented, and osteoclast apoptosis is stimulated. These drugs have affinity for active bone replacement sites and growth plates[30,31].

Bone resorption does not occur in patients undergoing bisphosphonate treatment due to inhibition by bisphosphonates of the osteoclastic activity that normally causes decreased bone replacement and a lack of new bone formation. In these patients, the bone is present for a longer period without replacement, making it prone to chronic infections and necrosis. Bisphosphonates also inhibit angiogenesis, leading to diminished bone vascularization and induction of bone cell apoptosis[27,32-34].

Osteonecrosis of the jaw is one of the complications of treatment with bisphosphonates in cancer patients. It most often occurs in the mandible and is associated with 53% of dental interventions; 48% of affected patients show a response to treatment when the drug has been used for a period of over eight weeks[35-37].

DIAGNOSTIC CRITERIA FOR BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW

Treatment with bisphosphonates must be considered in the medical history of patients with ulcerated lesions within the jaw with bone exposure for over eight weeks, necrotic bone, or lesions that do not heal spontaneously.

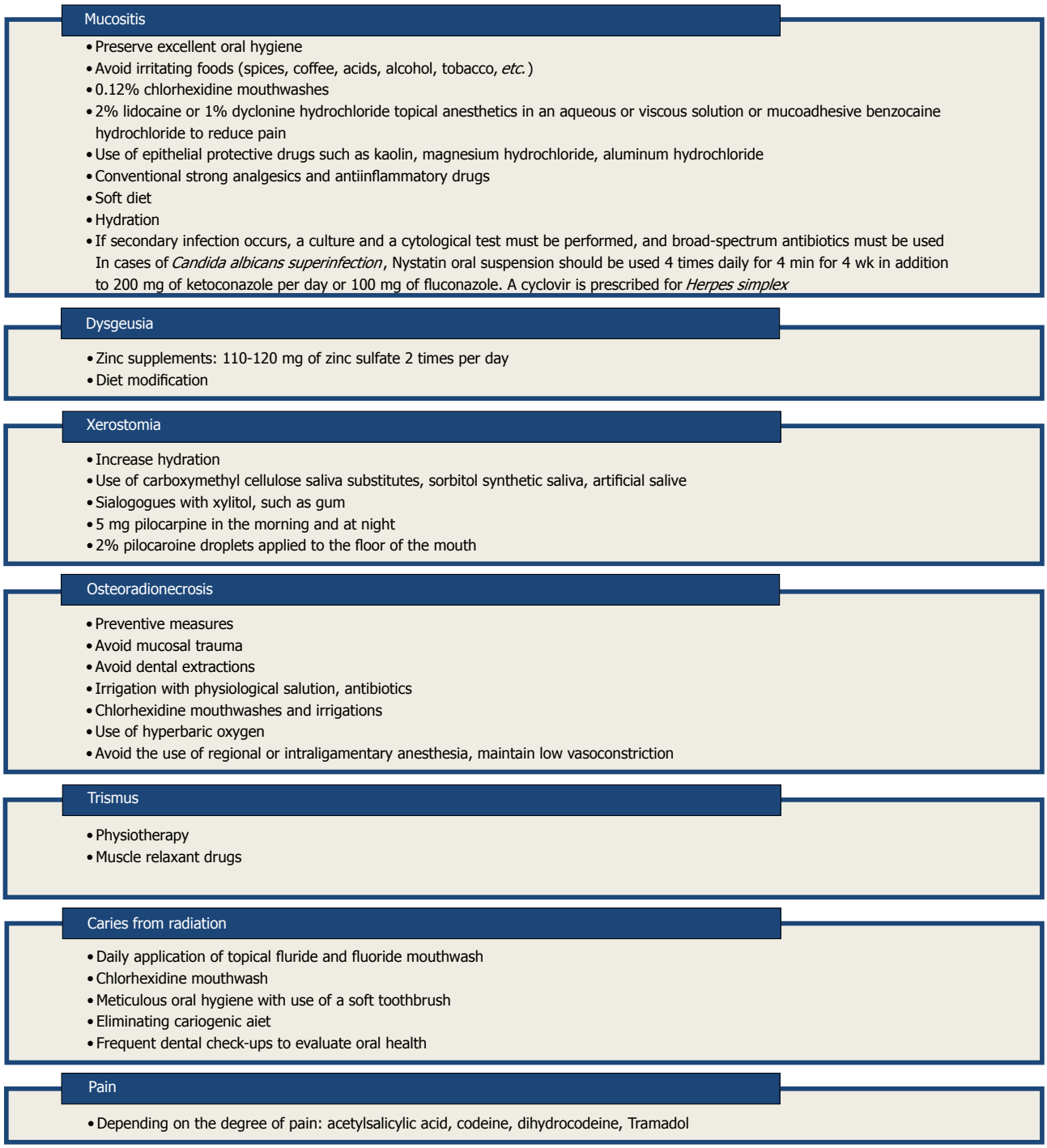

Figure 2 Treatment steps the dentist must follow in cases of oral complications in radiotherapy patients.

Additional tests such as orthopantomography and computerized tomography scanning are recommended to evaluate the extent of the lesion.

PREVENTIVE MEASURES IN PATIENTS UNDERGOING BISPHOSPHONATE TREATMENT

Once oral or intravenous treatment with bisphosphonates has been decided upon, preventive measures must be taken before and after treatment to avoid or minimize osteonecrosis[38,39].

Basic preventive measures prior to treatment focus on eliminating potential sites of infection and extracting teeth with poor prognoses to minimize the risk during therapy[40]. Once treatment has begun, meticulous control of the patient must be maintained so as to detect any sign of osteonecrosis. Good oral hygiene with plaque control and dental cleaning, tartar removal, and periodontal pocket treatment must also be included. Surgical procedures must be minimally invasive, performed only when

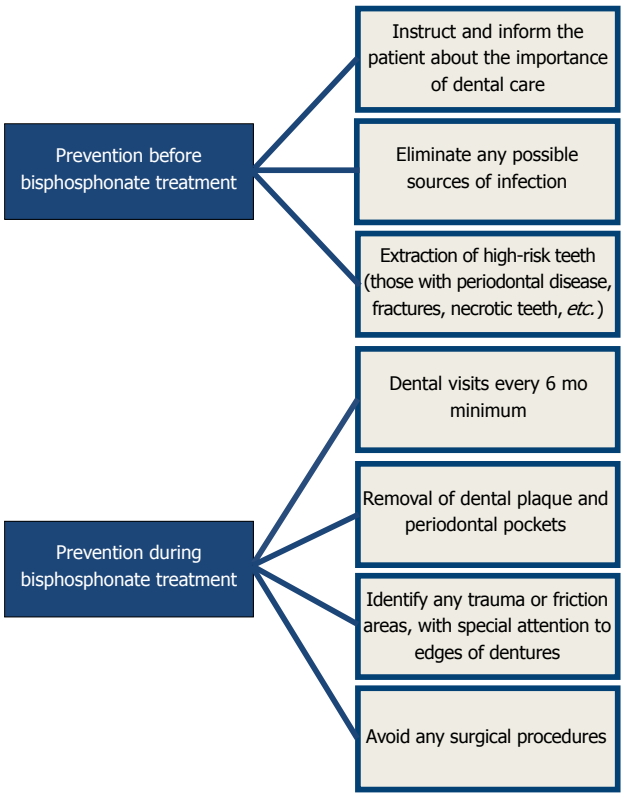

Figure 3 Preventive measures for bisphosphonate-related osteonecrosis of the jaw.

necessary, and include prophylactic antibiotic treatment and the use of chlorhexidine mouthwashes.

The Figure 3 outlines the preventive measures that must be followed before and after bisphosphonate treatment.

TREATMENT OF OSTEONECROSIS

The treatment of osteonecrosis consists of eliminating pain, controlling bone and soft tissue infections, reducing the progression of bone necrosis, decreasing or eliminating possible risk factors, improving oral hygiene, and following a specific antibiotic therapy protocol that is based on a previous culture growth and antibiogram of the exposed bone. If possible, bisphosphonate treatment should be suspended for 6 to 12 mo, which will allow improvement and possible resolution of the condition. Suspension of corticosteroid treatment is recommended when such agents are used as coadjuvants in the maintenance treatment. When conservative management fails, surgical debridement of necrotic bone tissue with a primary tension-free closure is an option, all with coadjuvant antibiotic prophylactic therapy[10,41-43].

Treatment depends on the size and extent of the lesion. Figure 4 outlines the treatment protocol depending on the extent of the lesion.

THERAPEUTIC INNOVATION AND TISSUE REGENERATION PERSPECTIVES

Tissues injured by chemo- and radiotherapy require

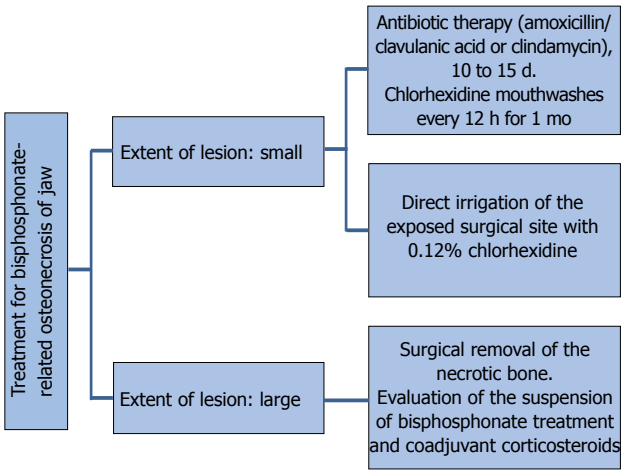

Figure 4 Therapeutic steps to be followed by the dentist in cases of bisphosphonate-related osteonecrosis of the jaw.

preventive measures to minimize damage. Furthermore, severe lesions with no possible repair require additional therapy. In this regard, the stem cell transplant described above is undergoing further development with the goal of achieving the regeneration of tissue damaged by radio- or chemotherapy.

Tissue and organ regeneration requires cells that can regenerate and that are similar to the cells that have been damaged, often irreversibly, by chemo- and radiotherapy. The possibility of restoring these cells allows consideration of tissue regeneration as a therapeutic option.

As the backbone of regenerative medicine, stem cells have acquired a decisive importance; scientific research in the field of stem cell biology has proven to be essential for allowing a transfer from basic to therapeutic research and generating new perspectives for clinical treatment.

Stem cells are defined as "cells that have both the capacity to self-renew (make more stem cells by cell division) and to differentiate into mature, specialized cells"[43]. Therefore, these cells provide a source of cells that can both generate other stem cells and form specific tissues and organs. Their absence limits or prevents regeneration.

Stem cell migration to the damaged site, as well as stem cell transplantation, are being considered as options in therapeutic regeneration, and currently, there are similar initiatives in radiotherapy[44,45]. Although at an experimental stage, the use of stem cells in such regeneration is potentially a viable therapeutic alternative. As indicated above, this approach could potentially be used to repair lesions caused by chemotherapy.

One of the therapeutic advantages of stem cell transplantation is the possibility of autologous transplantation; this would prevent the occurrence of graft versus host disease.

While research is progressing in this field, it must presently be considered an experimental field that, as such, does not permit the formation of any definitive conclusions.

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Molecular biomarkers of cell proliferation in ameloblastomas

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Abstract

Cell proliferation is a vital biological process that is important for all living organisms because of its role in growth and the maintenance of tissue homeostasis. The control of this important process differs greatly among benign and malignant neoplasms, and the evaluation of cell proliferation in neoplasms has become a common tool used by pathologists to provide useful information pertaining to diagnosis, clinical behavior, and treatment. The usefulness of information regarding cell proliferation has led to numerous studies on the value of these methods for diagnosing different types of tumors and for clinical decision making. Ameloblastomas are no exception. This review discusses the use of several classical molecular proliferation markers, including Ki-67, proliferating cell nuclear antigen, cyclin D1 and DNA topoisomerase Ⅱ alpha, to characterize ameloblastomas and proposes the use of new proliferation markers used previously to characterize other neoplasms. The use of these biomarkers offers valuable opportunities to evaluate the biological behavior of this type of odontogenic tumor.

Key words: Ameloblastoma; Ki-67; Proliferating cell nuclear antigen; cyclin D1; DNA topoisomerase

Core tip: Specific molecular markers are characteristic of particular cellular events such as proliferation, and in this context, "proliferation markers" refer to specific proteins or other factors in actively growing and dividing cells, whose presence serves as an indicator for such cells. In this mini-review, we aim to provide an overview of the methods currently available for the assessment of proliferation, and we review the different cell proliferation markers used to assess the biological behavior of ameloblastomas. In addition, we propose a new maker.

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INTRODUCTION

New cells are generated from pre-existing cells through an ordered sequence of events that is repeated. These events constitute the cell cycle. Traditionally, the cell cycle is divided into stages, the duration of which varies depending on the cell type.

The division of an original cell into daughter cells requires prior DNA replication and the synthesis of various proteins associated with this replication step, as well as the production of structures and organelles for the new cells. These processes occur at the interphase of the cycle, which itself is divided into phases: G1 (Gap 1), S

(Synthesis) and G2 (Gap 2).

When all the conditions necessary for division are met, M stage (or division) starts, which results in the separation of the chromosomes and then the division of the cytoplasm, known as cytokinesis. Some cells can remain in a state of active metabolism for a very long time without replicating their DNA or dividing. These cells are in G0 phase or the quiescent state. G0 is also considered a post-mitotic state[1].

The different cell types divide in a regulated manner. Certain environmental changes, such as temperature variations, changes in pH, nutrient scarcity and contact with neighboring cells, can slow down the cell cycle. Additionally, the presence of growth factors and hormones can trigger a series of intracellular processes that stimulate cell division. Cell proliferation can be defined as an increase in the number of cells as a result of cell growth and cell division.

In neoplastic processes, the abnormal and uncontrolled proliferation of cells is observed, and the cell cycle is altered. The assessment of cell proliferation activity in tumors has become a common tool used by histopathologists to provide useful information for assessing and predicting the behavior of tumors-that is, their likelihood of local recurrence, their metastatic potential, and the growth of metastases, and thus the likely duration of disease-free survival and survival to death[2]. Today, the most common method for evaluating proliferative activity is the use of immunohistochemical techniques.

Immunohistochemical staining is widely used in the identification of abnormal cells such as those found in cancerous tumors. This technique is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological specimen.

Specific molecular markers are characteristic of particular cellular events such as proliferation, and in this context, "proliferation markers" refer to specific proteins or other factors in actively growing and dividing cells, whose presence serves as an indicator for such cells[3].

Two requirements have been postulated for this type of marker: (1) the antigen should be continuously present during the cell cycle of all cell types; and (2) the transition to a nonproliferative state from any step of the cell cycle should be followed by a rapid disappearance of the antigen[4].

Odontogenic tumors constitute a group of heterogeneous lesions that range from hamartomatous or non-neoplastic tissue proliferations to benign and malignant neoplasms with variable aggressiveness.

Odontoma is the most common odontogenic tumor, but it is considered a non-neoplastic lesion. Ameloblastoma is the most common odontogenic neoplasm. According to the 2005 Histological Classification of Tumors of the World Health Organization, ameloblastomas are divided into four variants: solid/multicystic, extraosseous/peripheral, desmoplastic and unicystic. There exist several histological subtypes: follicular, plexiform, acanthomatous, granular and basal cell. Although ameloblastomas are classified as benign neoplasms, they can be locally invasive and destructive tumors of the jawbone. The molecular mechanisms that regulate cell growth and invasion in ameloblastomas are unknown. Determining the proliferative activity of ameloblastomas may provide important information regarding the appropriate treatment strategy.

In this mini-review, we aim to provide an overview of the methods currently available for the assessment of proliferation, and we review the different cell proliferation markers used to assess the biological behavior of ameloblastomas.

There are many methods for determining the level of proliferative activity in different types of tumors, including the analysis of the mitotic index, flow cytometry, silver staining (AgNOR), and immunohistochemistry techniques. The last two are the most widely used techniques to study ameloblastomas.

It is important to clarify that there are more specific and sensitive techniques for determining the presence of these proliferation markers such proteomics techniques which allow to know what proteins are present or absent in these tumors. Another technique quantitative, sensitive and highly specific is the real-time polymerase chain reaction that allows determining the expression levels of genes in the ameloblastoma.

Both techniques are more expensive and more laborious than the immunohistochemistry technique that despite having less specificity and sensitivity has the advantage of being able to display "*in situ*" the presence of proteins, important data for understanding how the tumor proliferates.

MOLECULAR MARKERS

Silver binding nucleolar organizer region

Several methods have been used for the identification of proliferating cells in tissue sections with the aim of using them as markers of impending malignancy. One among of these methods is the silver binding nucleolar organizer region (AgNOR) technique.

Nucleolar organizer regions (NORs) are segments of DNA that are closely associated with nucleoli, which contain the ribosomal DNA. These regions therefore contribute strongly to the regulation of protein synthesis. NORs are argyrophilic and can therefore be visualized using a silver staining technique, what has led to the use of the term AgNOR[2]. AgNOR staining is a simple one-step staining technique that overcomes the disadvantages of other techniques, such as the requirements for sophisticated equipment and technical expertise, high cost and long runtime[3,5]. The amount of AgNOR protein, estimated during interphase, can be used as a marker of cell proliferation and has prognostic value for several human cancers.

In the study by Seifi *et al*[6], the number of AgNOR dots in solid/multicystic ameloblastomas was found to be higher than that in unicystic ameloblastomas.

Coleman *et al*[7] reported that unicystic ameloblastomas lined with characteristic epithelium had a significantly lower AgNOR count than solid ameloblastomas, residual dentigerous cysts and keratocystic odontogenic tumors, and these authors concluded that AgNOR counts are not

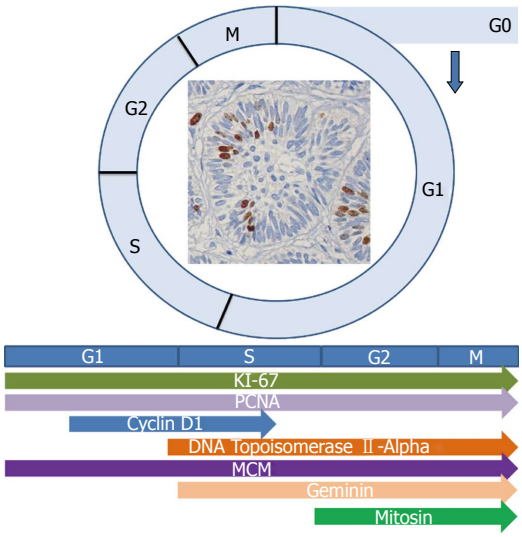

Figure 1 Presence of proliferation markers proteins during the cell cycle phases. The figure illustrates the presence of each marker of cell proliferation at different phases of the cell cycle. G1: Gap 1; G2: Gap 2; S: Synthesis; M: M-phase; PCNA: Proliferating cell nuclear antigen; MCM: Minichromosome maintenance complex.

of diagnostic significance and cannot be used to distinguish the various types of odontogenic cysts from one another or from unicystic ameloblastomas.

In terms of histological patterns, a significant difference has been found only between the follicular and plexiform types[8]. A significantly higher number of AgNOR dots per nucleus was found in follicular ameloblastoma cells than in plexiform ameloblastoma cells[9].

IMMUNOHISTOCHEMISTRY OF PROLIFERATION-ASSOCIATED ANTIGENS

Ki-67

The monoclonal antibody Ki-67 was first described in 1983 by Gerdes *et al*[10], who suggested that it might be used as a marker for proliferating cells. The Ki-67 antigen (Ki-67) is a classic marker of cellular proliferation and has been widely applied in the diagnostic, research and drug-discovery fields. The Ki-67 antigen was originally defined by the monoclonal antibody Ki-67, the name of which was derived from the city of origin (Kiel) and the number of the original clone in the 96-well plate[10]. The expression of Ki-67 occurs during all phases of the cell cycle except the G0 phase and the early G1 phase (Figure 1), and the expression level increases as cell proliferation progresses, especially in the S phase, with peaks in the G2 and M phases. This protein is then degraded rapidly after mitosis[3]. The standard antibody for the detection of Ki-67 is MIB-1. The fraction of MIB-1-positive tumor cells (the MIB-1/Ki-67 labeling index) is often correlated with the clinical course of the cancer, and Ki-67 is of prognostic value for many types of malignant tumors[11]. There have been numerous studies that have aimed to determine the proliferative capacity of ameloblastomas using the Ki-67 marker (Table 1). However, the comparisons of solid or multicystic tumors with unicystic tumors have yielded conflicting results. Some authors found a higher rate of positivity for Ki-67 in the solid/multicystic type[12], but other authors obtained different results, finding that the unicystic type had greater Ki-67 positivity[13-15]. Given that

Table 1 Cell proliferative activity measured using Ki-67 and/or proliferating cell nuclear antigen antibodies in ameloblastomas

Ref.	*n*	Type (*n*)	Ki-67	PCNA
Kim *et al*[40]	38	Unicystic (13)		+
		Solid/Multicystic (25)		
		Follicular		+
		Plexiform		
		Acanthomatous		
		Granular		
		Basal Cell		
		Ameloblastic Carcinoma		+
Fonaoka *et al*[41]	23	Plexiform (15)		++
		Follicular (5)		+++
		Unicystic (3)		+
Ong'uti *et al*[18]	54	Plexiform (30)	+	
		Follicular (24)	++	
Piatelli *et al*[42]	22	Unicystic (5)		+
		Solid/Multicystic (13)		
		Plexiform (5)		++++
		Follicular (4)		++
		Acanthomatous (4)		+++
Nagao *et al*[43]	30	Plexiform (15)	++	
		Follicular (15)	+	
Sandra *et al*[12]	32	Plexiform (9)	++++	++++
		Follicular (9)	+++++	+++++
		Acanthomatous (3)	+++	+++
		Basal Cell (3)	++++++	++++++
		Desmoplastic (3)	+	++
		Unicystic (5)	++	+
Han *et al*[19]	70	Follicular (ND)	+++	
		Plexiform (ND)	++	
		Unicystic (ND)	+	
Meer *et al*[15]	20	Solid/Multicystic (10)	+	+
		Unicystic (10)	++	++
Galvão *et al*[44]	16	Follicular (7)		++++
		Plexiform (4)		++
		Acanthomatous (3)		+++
		Basal Cell (2)		+
Bologna-Molina *et al*[3,13,45]	120	Solid/Multicystic (66)	+++	+++
	10	Unicystic (87)	++++	++++
	161	Peripheral (3)	++	++
		Desmoplastic (5)	+	+
		Ameloblastic Carcinoma (4)	+++++	+++++
Rizzardi *et al*[14]	15	Peripheral (2)	+++	
		Unicystic (2)	++	
		Solid/Multicystic (11)	+	
Salehinejad[46]	30	Plexiform (15)		+
		Follicular (12)		+
		Acanthomatous (3)		++
Yoon *et al*[47]	17	Ameloblastomas (10)	+	
		Ameloblastic Carcinoma (7)	++	
Maya *et al*[21]	15	Plexiform		+++
		Follicular		++
		Unicystic		+

The table describes the immunohistochemical studies performed with markers proliferating cell nuclear antigen and Ki-67 from the year 1994 to date. PCNA: Proliferating cell nuclear antigen.

several clinicopathological studies have found that solid ameloblastomas are more aggressive than unicystic ameloblastomas[16-19], the higher index of cell proliferation in unicystic ameloblastomas (determined using the Ki-67 antibody) found in some studies appears contradictory. This finding could be explained by the fact that unicystic ameloblastomas contain fewer stellate reticulum-like cells than solid/multicystic ameloblastomas, and consequently, most of the cells counted corresponded to basal or suprabasal layers, which are more likely to be positive. In other words, the proportions of the diverse types of epithelial cells, as well as the different mechanisms of growth in unicystic ameloblastoma and solid/multicysticameloblastoma , may influence the results of the proliferation index[13].

When histological subtypes were studied Ong'uti *et al*[18] in a study of 54 cases of ameloblastoma in Kenya, these researchers found that follicular ameloblastomas had a higher proliferation index than the plexiform variant. These results are similar to those of Han *et al*[19], who studied a Chinese population and found a slight predominance of a higher proliferation index in the follicular variant. Sandra and colleagues included the basal cell variant in their study, and this variant was found to have greater positivity for Ki-67 than the follicular variant[12]. In our previous study, we found similar results, with the follicular variant having a higher proliferation index[13].

Proliferating cell nuclear antigen

Proliferating cell nuclear antigen (PCNA) is a nuclear nonhistone protein that is necessary for DNA synthesis and is an accessory protein for DNA polymerase alpha, the expression of PCNA occurs during all phases of the cell cycle and the level of this protein is elevated during the G1/S phase of the cell cycle (Figure 1). PCNA expression can be used as a marker of cell proliferation because cells remain in the G1/S phase for a longer time when proliferating. Furthermore, this protein has an essential role in nucleic acid metabolism as a component of the DNA replication and repair machinery[20,21].

Multiple studies using PCNA have been performed to determine the rates of cell proliferation in various types of ameloblastomas, but the results are contradictory (Table 1). Some authors did not found any relevant differences between the different types and subtypes of ameloblastomas[3,11,22]. This result is most likely because PCNA is also involved in DNA repair. Because there is active ongoing DNA repair in many tumors, PCNA may also be upregulated in non-proliferating cells. Indeed, in some tumors, 100% of cells show positive staining. Therefore, after an initial period of popularity, PCNA is no longer considered a reliable proliferation marker in tumors[2]. Despite this conclusion, there are still numerous studies using PCNA as the first-choice marker of cell proliferation in ameloblastomas (Table 1).

Cyclin D1

The cyclins, together with cyclin-dependent kinases (CDKs), are the proteins responsible for the orderly progression of cells through the cell cycle. Cyclin D1 is amplified and/or overexpressed in a substantial proportion of different human tumors. Increased cyclin D1 expression occurs relatively early during tumorigenesis[23]. Changes in the genes encoding these proteins as well as changes in the expression levels of these proteins are found during the process of carcinogenesis. The overexpression of this protein leads to uncontrolled cell proliferation and tumor development[23]. Cyclin D1 is the regulatory subunit of the holoenzyme that phosphorylates and, together with sequential phosphorylation by cyclin E/CDK2, inactivates the cell-cycle inhibiting function of the retinoblastoma protein (pRb). pRb serves as a gatekeeper for the G1 phase, and passage through this restriction point leads to DNA synthesis. Thus, cyclin D1 promotes progression through the G1/S phase of the cell cycle (Figure 1)[24,25].

Follicular and plexiform ameloblastomas express cyclin D1 in many peripheral columnar or cuboidal cells and in some central polyhedral cells. No distinct difference in the reaction was detected between these two main tumor types[26]. Kumam *et al*[27] found that 19/25 follicular ameloblastomas were positive for staining with the cyclin D1 antibody, as were 9/10 plexiform ameloblastomas and 3/4 unicystic ameloblastomas.

DNA topoisomerase Ⅱ alpha

DNA topoisomerases are enzymes that disentangle the topological problems that arise from double-stranded DNA. Many of these problems can be solved by generating either single- or double-strand breaks. However, when it is necessary to alter the DNA topology by introducing transient double-strand breaks, only DNA topoisomerase Ⅱ (Top2) can fix the problem[28]. Type Ⅱ topoisomerases change the DNA topology by generating transient DNA double-strand breaks. The DNA topoisomerase Ⅱ alpha (TOP2α) is one of the major nuclear proteins, with peak expression in the S to G2/M phase. It is involved in nearly every aspect of DNA metabolism, playing an important role in chromosome organization and segregation[28].

Kumamoto *et al*[26] studied the presence of this protein in tooth germs and ameloblastomas, finding lower expression than that reported for Ki-67.

New cell proliferation markers

The usefulness of a marker for tumor diagnosis must be tested for each tumor type and application. Only those markers that have proven to be useful in practice should be considered. These three new cell proliferation markers have been studied in various types of cancer, although there are not currently any reports demonstrating their usefulness in ameloblastomas.

Geminin and minichromosome maintenance complex

Minichromosome maintenance complex (MCM2-7) and geminin have important roles in the prevention of DNA re-replication during the cell cycle. MCM proteins are

expressed cells in all phases of the cell cycle, including cells that exit the G0 and enter the G1 phase[29]. Geminin is present from the G1/S transition to the early M phase. Thus, MCM is a G0/G1/S/G2/M-phase marker, and geminin is an S/G2/M-phase marker (Figure 1)[30]. MCM proteins are known to contribute to the regulation of transcription, chromatin remodeling and checkpoint responses. The activated MCM complex appears to play a key role in DNA unwinding, acting as a DNA helicase[31]. Following the initiation of DNA replication during the cell cycle, geminin inhibits the reloading of the MCM complex onto chromatin and prevents DNA re-replication during the same cell cycle[32,33].

In recent years, these two proteins have been studied in various types of malignant neoplasms and have been shown to be very useful prognostic markers[34,35].

Mitosin

Mitosin, also termed centromere protein F (CENP-F), is a member of the human centromeric protein family. This protein is associated with the centromere/kinetochore complex and is expressed in all active phases of the cell cycle, with a maximum in G2 and M phases[36]. At the end of mitosis, CENP-F is rapidly proteolyzed by the proteasome. Accumulating evidence suggests that CENP-F is an important protein involved in chromosome alignment and kinetochore-microtubule interactions.

The cell cycle-specific expression of CENP-F makes it a potential marker of proliferation. Indeed, CENP-F is correlated with tumor proliferation in a variety of human tumors, including lung cancer[37], non-Hodgkin lymphoma[38] and salivary gland tumors[39].

CONCLUSION

The evaluation of cell proliferation activity in tumors provides useful information related to diagnosis, clinical behavior, treatment and research.

Note that the use of these biomarkers alone are not useful for the diagnosis of ameloblastoma, the diagnosis is based on clinical and histopathologic features, but yet proliferation molecular biomarkers provide important information when predicting the prognosis of patients with ameloblastoma, so the histopathological types together with proliferation marker expression could be useful tools for evaluating the biological behavior of ameloblastomas.

Over the past several decades, various cell proliferation biomarkers have been demonstrated to be useful in the study of various types of neoplasms, and these markers have been studied in some odontogenic tumors and ameloblastomas. The Ki-67 protein remains an excellent operational marker for determining the growth fraction of a given cell population and is considered the gold standard method for the evaluation of proliferation activity.

Despite the abundance of research, the results regarding which type of ameloblastoma has the highest rate of cell proliferation remain controversial. One problem is the lack of standardization regarding how to determine the cell count among many studies. In addition, ameloblastomas are polymorphic odontogenic tumors, with various types and variants, and the specific histomorphology of each type and the different mechanisms of growth may influence the observed proliferation index counting.

In recent years there have been found some new molecular biomarkers directly involved in the proliferation biology of tumors. Today new monoclonal antibodies are being tested in different tumors, hence the importance of new research using these new markers in ameloblastomas.

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BRIEF ARTICLE

Cytotoxicity of a silorane-based dental composite on human gingival fibroblasts

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Abstract

AIM: To evaluate the direct and indirect biocompatibility of Filtek Silorane on human gingival fibroblastic cells.

METHODS: Sixty-three standardized cylindrical specimens (8 mm diameter and 2 mm thickness) of restorative material were prepared using a light emitting diode-curing unit. The sample were built up in one increment and divided in 2 groups. In the first group, 21 samples (unpolished samples) were left without a specific polishing procedure; in the second one, 42 samples (polished samples) were polished with 4 different grains of discs. Fibroblast cultures, obtained from gingiva of 2 subjects without systemic and oral disease, were used to assess the direct and indirect biocompatibility. Cells cultured for 48 h in normal culture medium were used as a control.

RESULTS: The scanning electron microscope observations of fibroblasts cultured on the silorane samples, either polished or unpolished, confirmed the good biocompatibility of the material, favouring the cellular spreading. 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide tests showed a significant reduction ($P < 0.01$) of gingival fibroblasts viability cultured both in polished samples (90.05% ± 19.00%) and unpolished samples (78.15% ± 11.00%) compared with the control. Cells growth in medium conditioned with the samples for 1 wk showed a significant viability reduction ($P < 0.01$) compared to the control. A reduction of cell viability was observed even in the groups containing the material for 3 wk (polished: 89.45% ± 10.00%; unpolished: 65.97% ± 10.00%), even if the cytotoxicity was reduced after this long time exposure.

CONCLUSION: Although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class Ⅱ restorations

Key words: Silorane; Cytotoxicity; Resin composite; Fibroblasts

Core tip: The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material, thus decreasing the cytotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

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INTRODUCTION

Recently, the use of composite materials for restoring dental elements has significantly increased due to the growing aesthetic demand of patients[1].

Despite extensive improvements in mechanical and aesthetic properties of dental composites, volumetric shrinkage and contraction stress during polymerization are still a problem[1]. Contraction stress transferred to the tooth may lead to cusp deflection or enamel micro cracks; additionally, contraction stress of tooth-composite interface can determinate post-operative sensitivity, microleakage, marginal discoloration and recurrent caries[2].

In several studies different techniques have been investigated in order to minimize polymerization shrinkage and contraction stress[3-7]. At the same purpose low-shrinkage materials have been proposed, but none of them offered significant improvement to Bis-GMA-based composites[8].

In 2007, a low shrinkage dental composite based on silorane monomers has been introduced. This material contains traditional filler particles (quartz) and monomers based on a silane or a siloxane core bonded with several oxirane functional groups. The silorane monomers polymerize by a ring-opening polymerization process of the oxirane groups. According to its composition, this resin has two advantages: low polymerization shrinkage, due to the ring-opening oxirane monomer, and increased hydrophobicity, due to the presence of the siloxanes[9].

The release of substances from dental composite materials after polymerization and their possible toxicity have been widely examined during previous years[10-12]. Several *in vitro* studies have shown cytotoxic, genotoxic, mutagenic, or estrogenic effects of some monomers released by composite materials[13-17].

Limited information is available about the substance eluted from silorane composite and its cell or tissue compatibility. Kopperud *et al*[18] found no substance eluted from Filtek silorane in water, while silorane were found in ethanol solution. Krifka *et al*[19] revealed no significant signs of cytotoxicity on human pulp-derived cells caused by silorane-based materials, while a slight increase in reactive oxygen species was detected.

The aim of present study was to evaluate the biocompatibility of Filtek silorane. The maintaining of surface architecture after finishing was also investigated. These properties were investigated in polished and unpolished silorane polymerized samples.

As regards biocompatibility, we studied the viability of human fibroblastic cells both after direct contact with silorane composite and after cells conditioning using a medium exposed to silorane.

MATERIALS AND METHODS

Sixty-three standardized cylindrical specimens (8 mm in diameter and 2 mm in thickness) were prepared using a transparent plastic molds. The molds were positioned on a glass plate and filled with Filtek silorane (3 mol/L ESPE, Seefeld, Germany). The samples were built up in one increment. The specimens were polymerized using a diode unit with a power of 1100 Mw/cm^2 for 60 s (LE Demetron I; Kerr, Bioggio, Switzerland). Forty two of these samples were polished using a slow speed handpiece using 4 polishing discs of different grains (Sof-Lex discs, 3 mol/L ESPE; Seefeld, Germany), from the most (2382 C) to the least (2382 SF) abrasive. The remaining samples were left unpolished. All the samples were processed for observation under a scanning electron microscope (SEM: Philips XL20; FEI, Milano, Italy).

Cell culture

Cultured fibroblasts were obtained from subjects without systemic and oral disease, after signing informed consent. Biopsies (2 cm × 2 cm were taken from the gingiva of 2 subjects (40 years old), rinsed twice with phosphate buffered saline (PBS) at pH 7.4, containing penicillin (100 U/mL), streptomycin (100 μg/mL) and amphotericin B (2.5 μg/mL; all from Sigma Aldrich, Milan, Italy) and cut in small pieces with a sterile blazer. The tissue fragments were placed in culture flasks of 25 cm^2 with Dulbecco Modified Essential Medium (DMEM), containing 1 mg/mL of collagenase (all from Sigma Aldrich), and incubated for 3 h at 37 ℃. Afterwards, fragments were incubated at 37 ℃ (5% CO_2) in Petri plates of 35 mm containing DMEM supplemented with 10% of fetal bovine serum (FBS, Life Technologies, Monza, Italy), 4.5 g/L of glucose, penicillin (100 U/mL) and streptomycin (100 μg/mL) all from Sigma Aldrich. The first fibroblast cells were visible after 3-4 d. Culture medium was changed twice a week until cells confluence (2 wk). Using a trypsin/EDTA treatment (0.25% trypsin, 0.02% EDTA; Sigma Aldrich), the cells were detached and cultured in flasks of 75 cm^2 until a new confluence was achieved. Cells between the 2nd and the 4th passage of subculture have been used.

For direct toxicity test, silorane samples have been disinfected with alcohol at 70% for 3 h and washed with PBS for 24 h after the alcohol removing. After a conditioning treatment in DMEM containing 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL) for 24 h, the medium was discarded and samples considered suitable for cell seeding. Specimens were placed in ultra-low attachment 24/well plates (Corning, Tewksbury, MA, United States) and seeded with 1 × 10^4 cells/cm^2.

To assess indirect toxicity assay, samples disinfected as previously described were placed in agitation in DMEM containing 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL) for 1 and 3 wk. The conditioned medium was placed in contact with fibroblasts (1 × 10^4 cells/cm^2) seeded in 24/well polystyrene plates for 48 h. Cells cultured for 48 h in normal culture medium were used as a control.

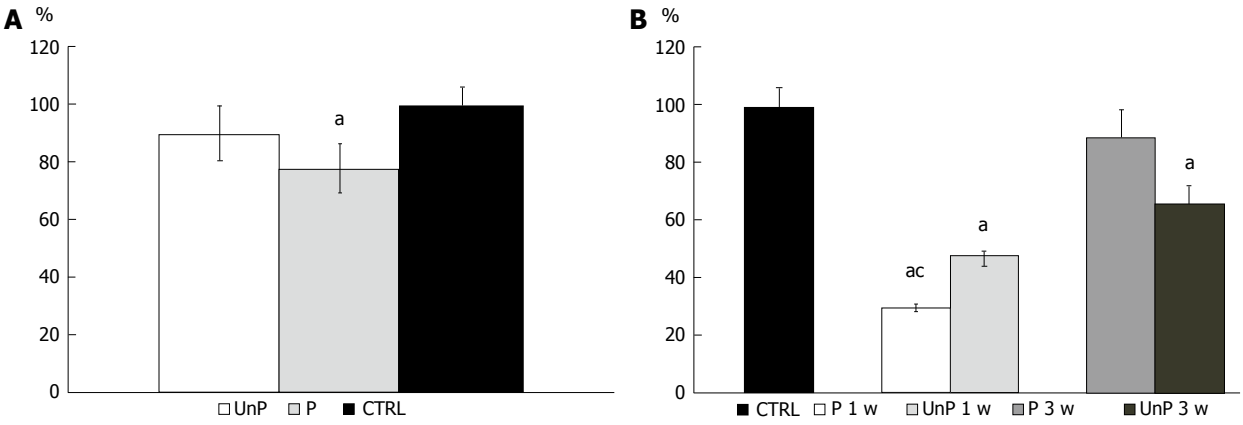

Figure 1 Histogram of cell viability. A: Cell viability of fibroblast cultured directly on unpolished samples (UnP), polished samples (P: finished surface using polishing discs) and control (CTRL); B: Cell viability of fibroblasts in CTRL, polished samples at 1 wk (P 1 w), unpolished samples at 1 wk (UnP 1 w), polished samples at 3 wk (P 3 w), unpolished samples at 3 wk (UnP 3 w); [a]$P < 0.05$ *vs* CTRL; [c]$P < 0.05$ P 1 w *vs* P 3 w.

Cell culture processing for SEM analysis

The obtained monolayer cells were fixed in 2% glutaraldehyde in cacodylate buffer for one hour at 4 ℃. After fixation, cells were rinsed in cacodylate buffer 0.1 mol/L, pH 7.4 and 7% sucrose; cells were then post-fixed using 0.1% OsO_4 in cacodylate buffer 0.1 mol/L, at 7.4 pH (1 h in dark at 4 ℃). After a second rinse in cacodylate buffer for 10 min, samples were dehydrated using a growing grade of ethanol (from 25% to 100%) at 4 ℃ with Critical Point Drying at 31.3 ℃ and 72.9 Atm. The samples were placed on aluminium stubs with a graphite-based glue, covered with gold, using an Edwards sputtering device, and observed with a SEM operating at 20 kV.

Cell culture processing for 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide test

After 48 h of culture, medium was removed and 200 μL of a solution (5 mg/mL in medium without phenol red) containing 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide (MTT; Aldrich, Sigma) and 1.8 mL of medium was added to the monolayer cells. The plates were incubated at 37 ℃ for 4 h. The supernatant was removed, the blue-violet formazan crystals were dissolved adding 2 mL of solvent (HCL 4% in isopropanol) and quantified with the spectrophotometer (Secoman; Anthelie light, 3.8 version, Contardi, Italia) at 570 and 690 nm. The results have been reported as viability percentage compared with the control culture.

Statistical analysis

Statistical analysis of the data was performed using two-ways analysis of variance. In detail, cell viability was evaluated on fibroblasts: (1) directly cultured on polished samples (P), unpolished samples (UnP) and control (CTRL); and (2) in contact with the eluates of P, UnP and CTRL samples at 1 and 3 wk.

Levels of $P < 0.05$ were considered to be statistically significant. The results were also evaluated in accordance with ISO standard 10993-5[20] which describes less than 25% inhibition as non-cytotoxic, 25% to 50% inhibition as slightly cytotoxic, 50% to 75% inhibition as moderately cytotoxic and more than 75% inhibition as highly cytotoxic[21].

RESULTS

Biocompatibility

MTT tests showed a significant reduction ($P < 0.01$) of gingival fibroblasts viability cultured both in P (90.05% ± 19.03%) and in UnP (78.15% ± 11.01%) compared with the CTRL (100.00% ± 6.00%), as shown in Figure 1A.

As regards to indirect toxicity, the viability of fibroblastic cells incubated in a medium conditioned with both P and UnP, for 1 or 3 wk, respectively, was studied using MTT test.

Cells growth in medium conditioned for 1 wk showed a significant viability reduction ($P < 0.01$) compared to the CTRL: the group conditioned with P showed a viability of 29.83% ± 1.92%, the one with UnP: 47.06% ± 1.87% (Figure 1B).

A reduction of cell viability was also observed in both groups conditioned for 3 wk (P: 89.45% ± 10.11%; UnP: 65.97% ± 9.89%), but only in the second group this reduction was statistically significant (Figure 1B).

SEM evaluation

As shown in Figure 2, SEM observations of fibroblasts cultured on the silorane samples, either P or UnP, confirmed the good biocompatibility of this material, which favoured cell spreading. These observations showed that the surface of the silorane-based material is able to absorb a big quantity of the serum component from the culture medium.

DISCUSSION

Silorane-based composite is a candidate for use in conservative dentistry due to its low polymerization shrinkage. However, it cannot be excluded that the potential release of remaining monomer substances may exert harmful effects on cells of periodontal tissues[22]. The current

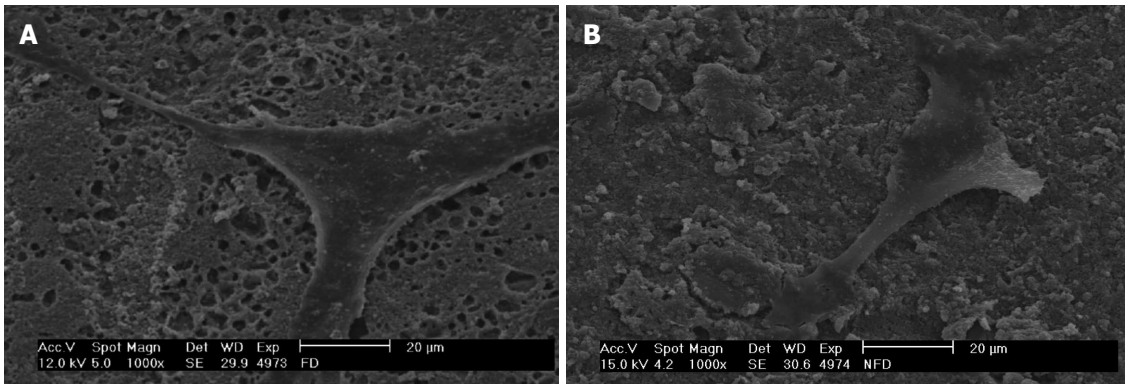

Figure 2 Scanning electron micrograph (x 2000, magnification). A: Gingival fibroblasts cultured directly on polished sample; B: Gingival fibroblasts cultured directly on unpolished sample.

limited literature indicates that silorane-based composite has a low toxicity presumably due to the low rate of free monomers released after polymerization[18]. In order to ensure a safe use of silorane-based materials, studies on the biocompatibility of this material are still needed.

Biocompatibility of a dental material can be studied exposing tissue directly to the material (direct toxicity) or placing it in a medium (conditioning), which will be used for additional tests (indirect toxicity)[23].

The results obtained in our study show a low direct cytotoxicity of both samples: P and UnP. The percentage of survival is lower in UnP than in P probably due to the larger surface contact area between composite and fibroblasts. Furthermore, the presence of oxygen inhibits the polymerization, resulting in a higher percentage of unreacted composite on the composite surface. Incomplete polymerization not only causes a decrease in the mechanical properties, but it can cause tissue reaction, as shown by Spangberg *et al*[24]. Composite finishing and polishing may indeed decrease the toxicity, as hypothesized in the study of Mohsen and Vankerchoven[25,26]. A moderate (with a few peaks of high toxicity) indirect cytotoxicity was observed in the samples placed in culture medium conditioned for 1 wk with silorane eluates (being the UnP slightly less cytotoxic than the P ones). Slight indirect cytotoxicity values were obtained for the samples placed in culture with medium conditioned for 3 wk. Under this condition, the fibroblast cultures show a different behaviour, since cell viability was slightly greater in case of contact with P than with UnP ones. These findings are in agreement with Sheridan *et al*[27], reporting that the cytotoxic effect of acrylic resin was greater after polymerization and decreased with time for many resins. The authors concluded that the longer a prosthesis is soaked, the less cytotoxic effects it is likely to have regardless of the denture base resin it is manufactured from[27]. Due to the not univocal data among P and UnP, the surface roughness does not seem to be a determining factor in the study of indirect toxicity. Indirect toxicity can be determined by release of substances from silorane as widely described in scientific literature[22].

Scanning electron micrographs allow observing the characteristic fibroblastic spreading. This is consistent with a study of Balcells *et al*[28], which states that the adsorption of serum proteins present in the culture medium is the first event that occurs when cells are seeded on a material and the adsorbed protein layer influences cell adhesion, spreading and proliferation.

In conclusion, although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class Ⅱ restorations[29]. The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material[30], thus decreasing the cytotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

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COMMENTS

Background

Despite extensive improvements in mechanical and aesthetic properties of dental composites, volumetric shrinkage and contraction stress during polymerization are still a problem.

Research frontiers

In several studies different techniques have been investigated in order to minimize polymerization shrinkage and contraction stress at the same purpose low-shrinkage materials have been proposed but none of them offered significant improvement to Bis-GMA-based composites.

Innovations and breakthroughs

The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material, thus decreasing the cytotoxicity after long time exposure.

Applications

Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

Peer review

The authors considered and concluded that the materials are biocompatible.

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BRIEF ARTICLE

Accuracy of linear *vs* spiral tomography: Alveolar crest to sinus/nasal floor height

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Abstract

AIM: To determine the accuracy of tomography in the linear measurement of alveolar bone at maxillary sinus/nose location.

METHODS: Two dry skulls each marked with 10 pairs of guttaperchas placed on buccal and lingual sides of the maxillary ridge were used in this *in vitro* study. The distance between the alveolar crest and the sinus/nasal floor was measured on tomographic views, prepared by linear and spiral techniques. The ridges were then sectioned so that each section would include one pair of buccal and lingual guttaperchas. The actual distances directly measured on the sections were compared to those of the equivalent tomographic sections (the magnification co-efficient was applied). Paired *t*-test was used to statistically analyze the data.

RESULTS: The measurement error with the application of linear tomography and spiral tomography was shown to be 0.455 ± 0.838 mm (P = 0.029) and 0.17 ± 0.78 mm (P = 0.347), respectively. There was a statistically significant difference between the liner tomography values and actual values (P = 0.029). This difference was representative of underestimation. McNamara's test was used to assess the ± 1 mm error; 73.7% of the linear values and 84.2% of the spiral values were within the ± 1 mm error limit. McNamara's test did not show any significant differences between the 2 methods in this regard (P = 0.625). The linear values were significantly different to the actual values (P = 0.029) but not to the spiral values (P = 0.185).

CONCLUSION: Spiral tomography has enough accuracy for the measurement of alveolar ridge height. Although linear tomography somewhat underestimates the actual values it provides satisfactory accuracy.

Key words: Linear tomography; Spiral tomography; Maxillary sinus; Dental implants

Core tip: Maxillary partial or complete edentulism represent some challenging conditions in implant dentistry. The position of sinus/nasal floor in partial/complete edentulous maxilla determines the alveolar bone height and consequently the length of the implants that can be used. Although cone beam cone beam computed tomography and conventional computed tomography are widely used for pre-operative implant treatment planning, they are expensive and can expose patients to relatively high dose of radiation. We demonstrated that tomography can be a good substitute for conventional and cone beam computed tomography for alveolar length measurement at maxilla, although spiral tomography is more accurate than linear tomography.

Accuracy of linear *vs* spiral tomography: Alveolar crest to sinus/nasal floor height. *World J Stomatol* 2013; 2(4): 91-96 Available from: URL: http://www.wjgnet.com/2218-6263/full/v2/i4/91.htm DOI: http://dx.doi.org/10.5321/wjs.v2.i4.91

INTRODUCTION

Although computed tomography (CT) and cone beam computed tomography (CBCT) are frequently used for pre-operative implant planning, their use in the post-operative assessments is limited due to metallic streak artifacts[1,2]. Also CBCT is associated with artifacts such as truncated view and beam hardening artifacts[3,4]. Another disadvantage of computed tomography is its relatively high radiation risk compared to conventional tomography[5-7].

The position of the maxillary sinus floor influences the height of the alveolar bone and consequently the implant length to be placed. Tomographic views are considered as the most reliable projections in the assessment of potential implant sites prior to surgery since they provide the clinician with the buccolingual information of the anatomic structures[1,5].

The measurement accuracy of most recent tomographic techniques in the assessment of mandibular landmarks has been thoroughly discussed through the literature[8-14]. A recent systematic review has concluded that each landmark possesses a unique error pattern and contributes independently to the measurement inaccuracy[15]. Since the alveolar crest height meaning the distance between the maxillary sinus floor and the alveolar crest serves as an important factor in the placement of dental implants., The present study aimed to compare the measurement accuracy of two tomography techniques, linear and spiral, in the maxilla of dry human skull.

MATERIALS AND METHODS

In this *in vitro* study, two dry skulls with intact maxillary sinus, nasal floor and foramen magnum, one completely and the other partially edentulous, were used. Each skull was then marked in 10 regions posteroanteriorly. Panoramic scout views were prepared to measure the alveolar ridge height. Each skull was then marked in five regions on each side with 70 guttapercha using glue every 1 cm and perpendicular to the ridge. The most distal guttapercha was placed in the third molar region and the most anterior one was placed in the lateral incisor region. A total of 20 areas (4 × 5) were marked (Figure 1). Each area was marked with two guttaperchas one on the lingual and the other on the buccal aspects of the alveolar ridge.

To prepare tomographic views, skulls were fixed on a wooden jig. Tomographic views were obtained twice, first using multitask Cranex TOME device (Orion Corporation Sordex, Helsinki, Finland) and then using Planmeca Promax (Helsinki, Finland). Kodak X-Omat cassette (Ektavision) and Agfa Single Emulsion (CP-VB) (15 cm × 30 cm) film were used. For spiral tomography, the dental tomo program for the upper jaw was selected at 57 kV, 2.5 mA and 56 s. Slice thickness was set to be 2 mm and the aperture number was 4. For linear tomography, the minimum adjustments, 54 kV and 0.5 mA, were set. Slice thickness was set to be 3 mm (Table 1). In all cases skulls were placed so that the maxillary occlusal plane would be parallel to the horizon. A total number of 20 cross-sections were prepared on each X-ray unit.

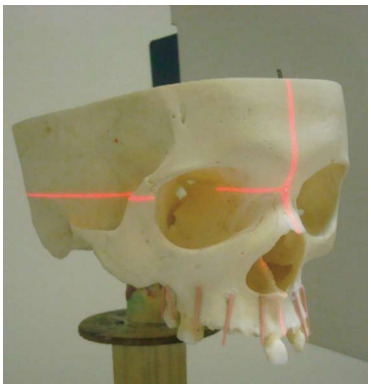


Figure 1 One of the two dry skulls marked with guttaperchas.

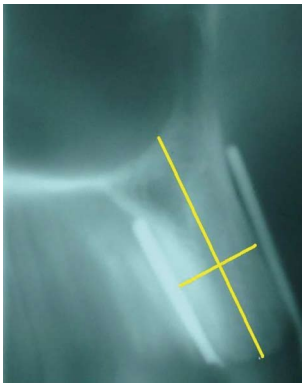


Figure 2 The measurement approach for bone height on film.

The films were processed in an automatic processing machine (OPTIMAX 2010; PROTEK Medizintechnik, oberstenfeld, Germany). Measurements were done on a negatoscope in a semi-dark room using a digital sliding caliper. The view with the clearest gutta-percha was selected for measurement on each radiograph. The alveolar crest and the maxillary/nasal floor were then outlined on tracing papers which were superimposed on the radiographic views. Bone height was measured in an oblique direction along the medial axis of the alveolar process, similar to the direction of implant placement[5]. The distance between the sinus floor to the alveolar ridge traced on this line was considered as the ridge height (Figure 2). Measurements were done twice by an oral radiologist and an oral radiology senior resident each with a time interval of 2 wk.

The whole alveolar process was cut with electric saw first and then hand jig saw was used to separate the

Figure 3 Sectioned specimen using a hand jig saw.

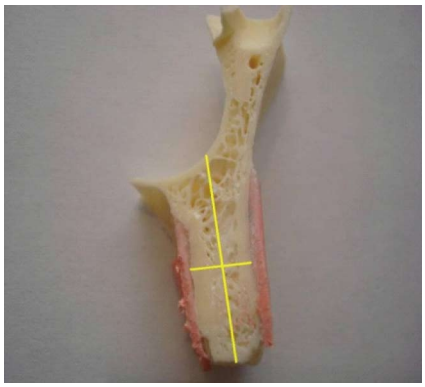


Figure 4 The measurement approach on the sectioned specimen.

Table 1 Summary of image protocols

Machine type	Promax	Cranex Tome
Manufactorer	Planmeca, Helsinki, Finland	Sordex, Helsinki, Finland
Tube voltage (kV)	54	57
Tube current (mA)	0.5	2.5
Slice thickness (mm)	3	2

marked sections (Figure 3). Bone height on the sections was measured similar to the films sing a digital sliding caliper with a nominal resolution of 0.01 mm (Figure 4). To determine the magnification factor, a pilot study was designed and performed through which the actual length of the guttaperchas and their length on the radiographic view was measured. The magnification was calculated dividing the mean radiographic values to the actual values. The magnification factors were similar to the ones defined by the manufacturer (1.5 for both devices). The actual values measured on the bone sections were considered as the gold standard.

Statistical analysis

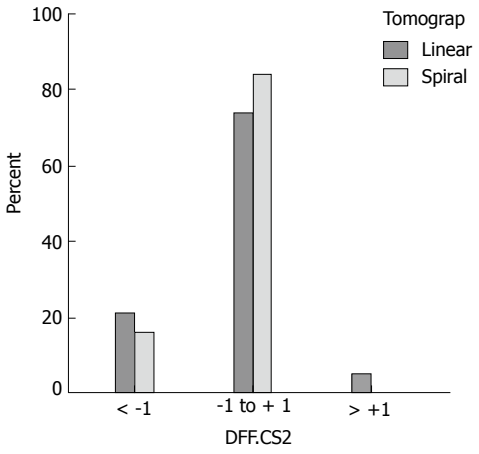
Data were inserted in SPSS v. 15, and then were analyzed by Paired *t*-test and McNemar's test. Linear regression model was used to assess the relation between the actual values and tomogram values. Mean values and standard deviations were used to describe quantitative values and percent. Proportions and bar charts were used to describe qualitative data. A 0.05 level of significance was considered.

RESULTS

Twenty specimens were primarily used in the present study, from which one specimen was excluded due to the displacement of guttapercha. The extent of maxillary sinus floor and nasal floor was recognizable on all tomographic views. Measurements were, however, more challenging in the posterior areas and also in dentate skull. Paired t-test was used to compare the measurement differences between the linear and spiral tomography to the actual values multiplied by the magnification co-efficient. The mean error for linear and spiral tomographic views were 0.455 ± 0.838 mm (P = 0.029) and 0.174 ± 0.787 mm (P = 0.347), respectively. There was a statistically significant difference between the linear tomography values and actual values (P = 0.029). This difference was representative of underestimation. Non-parametric Wilcoxon signed rank test (a non-parametric equivalent to paired *t*-test) also revealed a statistically significant difference for linear tomography (P = 0.035) and a statistically not significant difference for spiral tomography (P = 0.587).

Paired *t*-test also showed a significantly higher deviation from the actual values in linear tomography compared to spiral tomography (P = 0.017). This was confirmed by Wilcoxon test (P = 0.026). However, neither *t*-test (P = 0.185) nor Wilcoxon test (P = 0.199) revealed a significant difference between the linear and spiral values after they were multiplied by the magnification factor. McNemar's test was used to assess the ± 1 mm error. Error values in linear and spiral tomographies were within ± 1 mm respectively in 73.68% and 84.2% of the cases. McNemar's test did not show any significant differences between the two methods in this regard (P = 0.625).

After the application of magnification factors to the values obtained by linear tomography, overestimation and underestimation were respectively seen in 21.01% and 78.99% of the cases. Overestimation and underestimation were respectively seen in 47.3% and 52.7% cases of spiral tomography. All the overestimation cases of



Error	< -1 mm	-1 to +1 mm	> +1 mm
Linear	4 (% 21.01)	14 (% 73.68)	1 (% 5.31)
Spiral	3 (% 15.8)	16 (% 84.2)	0 (% 0)

Figure 5 Frequency percent of the measurement errors after the application of magnification factors.

spiral tomography were within the error limit of ± 1 mm. Mean spiral overestimation was within 0.02 to 0.91 mm. Mean linear overestimation was within 0.38 to 1.5 mm. Mean underestimation values of linear and spiral tomographies were respectively within the ranges of 0.04 to 1.8 mm and 0.03 to 1.4 mm (except for one case of spiral tomography which showed an underestimation of 2.19 mm). Figure 5 represent the distribution of errors within the ± 1 mm range.

DISCUSSION

Linear and spiral tomography techniques were compared in the present study in terms of accuracy in the preoperative assessment of the maxillary bone height. To the best of our knowledge, the literature lacks enough studies on the localization of maxilla, maxillary sinus and nasal floor and most studies have addressed the localization of mandibular canal.

It is generally accepted that more complex tomographic movements are associated with higher blurring of the background images and less streaking artifacts[1]. Spiral and hypocycloidal movements will reduce the incidence of streaking artifacts[16,17]. In the studies of Lindh *et al*[18] spiral tomography provided more accurate views of the mandibular canal compared to hypocycloidal tomography. In the present study, the actual values measured directly on the skull sections and the values obtained from linear tomograms were significantly different (P = 0.029). The mean difference was measured 0.455 ± 0.83 mm for linear tomography and 0.174 ± 0.78 mm in spiral tomography. This difference was not significant for spiral tomography (P = 0.347). The significant difference in linear tomography may be partly due to the quality of images. In spiral tomography the quality of images especially in the anterior area was better and the outlines could be more easily detected. Due to the insufficient number of specimens, however, this comparison between the anterior and the posterior areas was not statistically implacable.

Naitoh *et al*[19] compared the measurement accuracy of direct laser positioning and reformatted CT. They suggested that other factors including tomographic angle and the placement angle of the object to be projected also influence the measurement accuracy in addition to the motion pattern. They did not find any significant differences between the two methods in mandible measurements (P = 0.526). They believed the significant lower accuracy of the linear tomography found through other studies is due to the difficulties in the adjustment of the projection plan of the object and not due to the image quality.

The main purpose of the present study was to compare two types of tomography in terms of linear measurements rather than image quality. Statistical analysis failed to reveal any significant differences between the two methods in the measurement of the distance between the alveolar crest and the sinus/nasal floor (P = 0.185). Spiral tomography values obtained through the present study are consistent to the findings of Bou Serhal *et al*[5] who assessed the measurement accuracy of the spiral tomography in upper jaw. They reported a mean error of 0.24 ± 0.19 mm which was not significantly different to the actual values (P > 0.05). They stated some quality impairment in the images obtained from the most distal slices and though it was attributed to the placement of more bony structures in the area. Similarly, Kim *et al*[20] experienced quality impairment in mandibular posterior areas projected by Scanora spiral tomography. Higher image quality in the study of Bou Serhal *et al*[5] may be attributed to the complete edentulousness of the studied skulls, which may have eliminated the artifacts commonly created by the presence of restorations and natural teeth. Similarly, the quality of images obtained from the anterior areas was higher compared to those of posterior areas in the present study both with spiral and linear tomographies. Also spiral projections were associated with higher image quality in anterior areas compared to linear tomography.

Bou Serhal *et al*[21] also evaluated the accuracy of conventional spiral tomography [Cranex TOME multifunctional unit (Orion Corporation Sordex; Helsinki, Finland)] for the localization of the mandibular canal on human fresh cadavers and reported higher mean error values compared to their previous study. They concluded that the information provided by spiral tomography of the posterior mandible using the studied unit is reliable and sufficient for preoperative planning of implant placement. They attributed the different results of the two studies to the fact that the absence of overlying soft tissue provides the observer with a higher resolution of the bony mandible and also more precise adjustments of skull compared to the patient or cadaver in the latter study.

Butterfield *et al*[17] examined linear tomography in terms of accuracy and validity in the pre-surgical evaluation of potential implant sites in mandible. They claimed that linear tomography suffers from prominent dimensional instability, which significantly limits its role in

preoperative assessment of implant sites. Seven observers traced eight anatomic landmarks including the mandibular cortical bone and inferior alveolar canal on linear tomographic images. Statistically significant differences were found between the perceived and actual anatomic values ($P < 0.05$). They suggested that since the source to image receptor distance, source to object distance and object to image receptor distance change with a constant proportion to each other during the tomographic movements, linear tomography does not hold a constant magnification factor. Consistently, linear tomography in the present study was associated with a significant underestimation of the measurement values of alveolar crest height ($P = 0.029$). On the other hand, the pilot linear tomography of guttaperchas conducted by the author of the present study revealed a magnification factor (1.498) which closely approximated the manufacturers (1.5).

Bou Serhal *et al*[21] measured an actual magnification factor of 1.49 for the Cranex TOME spiral tomography unit both in vertical and horizontal planes. Closely similar, the magnification factor measured in the present study for the same device was equal to 1.518.

In the present study, spiral tomograms showed underestimation and overestimation respectively in 52.7% and 47.3% of the cases. The overestimation in the present study was higher than that of Bou Serhal study in 2000 (33.3%). Also the linear tomograms of our study presented with underestimation and overestimation in 78.99% and 21.01% of the cases, respectively. Based on these findings, it may be suggested that overestimation occurs more frequently in spiral tomography and underestimation occurs more frequently in linear tomography. Underestimation would seemingly be preferable in the implant placement especially when the mandibular body above the mandibular canal is considered as a potential implantation site[18]. Loubele *et al*[3] comparatively assessed the measurement accuracy of multi-slice spiral CT, spiral tomography and cone-beam tomography. They measured an overestimation of 1 mm for the spiral tomography. CBCT in their study (except for one case) was only associated with approximate overestimation of 0.5 mm. In the present study, spiral tomography was associated with an overestimation of less than 1 mm (maximum of 0.91 mm). This value was measured to be maximally 1.5 mm with the application of linear tomography especially in the anterior areas of dentate skulls. However, since the significant difference tended towards underestimation with the application of linear tomography in the present study, and also due to the higher safety of underestimation compared to overestimation, the measurement accuracy of the linear tomography seems to be within the satisfactory clinical range. On the other hand, underestimation may result in the application of a shorterimplant, which may impair the long term prognosis survival of the implant and success of the overlying prosthetic restoration[18].

Spiral tomography in the study of Bou Serhal *et al*[5] was associated with a accuracy of ± 1 mm in the measurement of alveolar crest to maxillary sinus distance. Klinge *et al*[22] measured the distance between the alveolar crest to the inferior border of the mandibular canal by means of hypocycloidal tomography and reported that only 39% of the cases were associated with a accuracy of within ± 1 mm. Hanazawa *et al*[23] measured the same distance by spiral tomography and reported that 47.9% of the cases are within the ± 1 mm accuracy. In the present study, spiral tomography and linear tomography were associated with ± 1 mm accuracy respectively in 84.2% and 73.68% of the cases after the magnification factors were applied.

McNemar's test did not reveal any significant differences between the two tomographies in terms of the percent of the cases within the ± 1 mm accuracy ($P = 0.625$). Clinically the value of underestimation and overestimation is more important than the absolute difference of the perceived and actual values[21].

The values measured on the linear tomograms obtained by Promax unit significantly differ to actual values ($P < 0.029$). This difference tends toward underestimation. There is, however, a significant correlation between the linear and spiral tomographies. Therefore it seems that both linear (Promax) and spiral (Cranex Tome) tomography are associated with sufficiently accuracy in the pre-surgical assessment of potential implant sites in dry skull. Also the magnification factor introduced by the manufacturer is seemingly reliable for both devices. Linear and spiral tomography did not show any significant differences in the present study. This may not be the case in clinical situation where the soft tissue and dentition are present. Therefore, studies on human cadaver or *in vivo* trials are highly recommended to further assess the reliability of the two methods.

COMMENTS

Background

Maxillary Partial or complete edentulism is a common condition in dentistry and insufficient bone can be a challenge for a clinician who wants to place implants at edentulous Maxilla. The loss of maxillary teeth result in decrease in bone height (and width). The position of the maxillary sinus floor/nasal floor influences the height of the available alveolar bone and consequently the implant length to be placed. Different imaging modalities are used for detemining the hight of available bone at maxillary sinus area.

Research frontiers

The imaging modality should be chosen that yields the necessary diagnostic information and results in less radiologic risk. Periapical radiography is of limited value in determining bone quantity due to its magnification, distortion, lack of third dimension and size limitation. Nowadays computed tomography and cone beam computed tomography are frequently used for pre-operative implant planning. Although they can provide us with invaluable information (3D images with high accuracy), they have their own disadvantages such as exposing the patient to the relatively high radiation, beam hardening artifacts and high costs. The hot spot is how to employ a modality which produces 3D images but wouldn't expose patients to a high radiation dose. The answer can be tomography.

Innovation and breakthrough

To the best knowledge of the authors of this article, the literature lacks enough studies on the localization of maxillary sinus and nasal floor and most studies have addressed the localization of mandibular canal. Linear and spiral tomography techniques were compared in the present study in terms of accuracy in the pre-operative assessment of the maxillary bone height. In this study, the actual values measured directly on the skull sections and the values obtained from linear

tomograms were significantly different. This difference was not significant for spiral tomography. The significant difference in linear tomography may be partly due to the quality of images. In spiral tomography the quality of images especially in the anterior area was better and the outlines could be more easily detected. Due to the insufficient number of specimens, however, this comparison between the anterior and the posterior areas was not statistically implacable.

Application

The study result suggests that spiral tomography has enough accuracy for the measurement of alveolar ridge height. Although linear tomography underestimates the actual values, it seems to provide satisfactory accuracy.

Terminology

Tomography is generic term for describing sectional radiography. X-ray source and film move in opposite direction during exposure in this technique. Consequently, structures in the section of interest are sharp while the above and blow sections appears blurred.

Peer review

This is an interesting study in which authors tested the accuracy of the measurements of linear and spiral tomography in maxilla. The results were intriguing and demonstrated that spiral tomography is an accurate imaging modality for pre-operative treatment plan. Although, linear tomography is not perceived as accurate as spiral tomography, it appears to be accurate enough to be utilized for a pre-surgical treatment plan.

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CASE REPORT

Surgical obturator duplicating original tissue-form restores esthetics and function in oral cancer

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Abstract

Oral cancer treatment primarily focused on the surgical removal of cancer tissues followed by surgical/prosthetic reconstruction. Restoration of the missing structures immediately after surgery shortens recovery time and allows patient to return to community as a functioning member. The most practiced surgical obturators are simple resin prosthetic bases without incorporation of the teeth. This article highlights a technique to fabricate a surgical obturator that duplicates patient's original tissue form including teeth, alveolus and palatal tissues. The obturator is placed immediately after surgery and make patient feel unaware of surgical deformity. The obturator prosthesis fabricated with this technique supports soft tissues and minimizes the scar contracture. We have clinically tried this technique in 11 patients. Patients' satisfaction level was recorded on visual analogue scale (VAS) and it ranges between 74% and 94% (with average of 87%). Four different prosthodontists have visually evaluated facial asymmetry of patients at 6 mo recall and their average perception on VAS varies between 71% and 93% (with average of 84%).

Key words: Maxillofacial prosthesis; Maxillectomy; Obturator prosthesis

Core tip: This article highlights a technique to fabricate a surgical obturator that duplicates patient's original tissue form including teeth, alveolus and palatal tissues. Make patient feel unaware of surgical deformity. The obturator prosthesis fabricated with this technique supports soft tissues and minimizes the scar contracture. We have clinically tried this technique in 11 patients. Patients' satisfaction level was recorded on visual analogue scale (VAS) and it ranges between 74% and 94% (with average of 87%). Four different prosthodontists have visually evaluated facial asymmetry of patients at 6 months recall and their average perception on VAS varies between 71% and 93% (with average of 84%).

Patil PG. Surgical obturator duplicating original tissue-form restores esthetics and function in oral cancer. *World J Stomatol* 2013; 2(4): 97-102 Available from: URL: http://www.wjgnet.com/2218-6263/full/v2/i4/97.htm DOI: http://dx.doi.org/10.5321/wjs.v2.i4.97

INTRODUCTION

Treatment of oral cancer necessitates surgical removal of the affected maxillofacial hard and soft tissues[1]. Loss of structural continuity affects esthetic appearance and functional performance like mastication and swallowing[2]. Esthetic disfigurement significantly affects patients' social and psychological wellbeing[3]. In addition to local and general health psychological, social and economic aspects determine final treatment outcome of the prosthetic rehabilitation[2]. Patient may get disease free after resection of the cancer tissues but can become a permanent handicap if not rehabilitated in a proper manner. Plastic surgeons face great challenges to reconstruct oral and maxillofacial defects to maintain the functional integrity and esthetic appearance especially in large sized defects and trismus. In

case of malignancies, radiotherapy is a vital parameter in controlling neck metastasis. Though surgical reconstruction restores the defect, replacement of teeth and facial tissue support can only be achieved by prosthodontic reconstruction. Obturators are given to maintain an artificial barrier between nasopharynx and oropharynx so that oral intake of food should not be regurgitated from the nose and sufficient negative pressure develops in the oral cavity to facilitate deglutition[1]. Depending upon the time of prosthesis given they are classified as immediate surgical (immediate), delayed surgical (7-10 d), interim (4-6 wk) and definitive (After 4-6 mo) obturators[1,4].

Immediate or delayed surgical obturator minimizes scar contracture and disfigurement thereby making a positive effect on the patients' psychology. Various designs have been proposed for fabrication of the surgical obturator. The design ranges from acrylic resin record base bearing no teeth[5]. With or without wrought-wire clasps[6], to a clasped acrylic resin prosthesis that restores the dental arch form[7]. Dentate patients are relatively easier to treat than edentulous patients as maximum retention and stability can be achieved from remaining teeth. The most practiced surgical obturator is a simple resin prosthetic base without incorporation of teeth. Addition of teeth in initial healing phase may cause constant source of irritation and hamper healing process according to many authors. However only anterior teeth can be restored until surgical wound is healed in some clinical situations[1,8]. In case of radiotherapy, the tissues become more friable and vulnerable hence the simplest form of prosthesis is advocated in most of the clinical situations[3].

This article highlights a treatment concept which restores resected maxillofacial tissues with a surgical obturator that duplicates patient's original tissue form[9,10]. The technique is developed in our hospital and total 11 cancer patients were treated in last 2 years. Patients were evaluated for patients' satisfaction level and clinicians' perception for bilateral facial symmetry on visual analogue scale (VAS) at 6 mo recall visit.

Technique

Prior to surgery maxillofacial prosthodontist should examine patient and discuss the plan of treatment with surgeons about a proposed line of incision and amount of resection (Figure 1A).

A pre-surgical impression of the maxillary arch is made with irreversible hydrocolloid and poured to obtain a working cast. An anticipated line of resection is drawn with a marking pencil on the cast following discussion with maxillofacial and plastic surgeons.

The area (of the legion) on the cast is modified to obtain normal anatomical contours (Figure 1B). For example, swollen areas of the lesion on the cast can be scraped-out and defect (ulcer/breach) areas can be built-up with dental stone in order to create the normal anatomical tissue form on the cast.

Labial/lingual infrabulge retentive areas of the remaining healthy teeth are engaged with the retentive clasp arms. A processed prosthetic base is fabricated in heat polymerizing acrylic resin by incorporating the clasps (Figure 1C).

The processed prosthetic base is reseated on the maxillary cast and an over-impression of the whole cast (along with the seated processed prosthetic base) is made with polyvinyl-siloxane putty in perforated stock metal tray to form putty impression index (PⅡ) (Figure 1D). Facial surface on the defect side of the cast should be completely recorded in the over-impression till border areas.

The PⅡ and the cast are separated from each other. The prosthetic base is reseated on the PⅡ (Figure 1E).

The separated cast is sectioned according to the anticipated line of resection. The planned defect section of the cast is separated from the remaining normal portion (Figure 1F). This remaining portion (of normal structures) of the cast is used to fabricate the prosthesis.

The PⅡ (along with the processed prosthetic base) is reseated onto the remaining portion of the cast (Figure 1G).

Prosthetic teeth are created with sprinkle-on technique by incrementally adding tooth-colored autopolymerizing acrylic resin into the impression areas of teeth in the PⅡ. The facial flange can also be created by adding the pink colored autopolymerizing acrylic resin uniformly 2-3 mm in width (Figure 1H).

After setting the PⅡ along with the prosthesis is separated from the cast (Figure 1I) and then PⅡ is separated from the prosthesis carefully. The excess resin is removed and the flange and teeth areas are finished and polished in a conventional manner (Figure 2)[11,12] Note that the smooth borders and polished surfaces are critical parameters to avoid any tissue injury. Occlusal surfaces of the posterior teeth can be trimmed off by approximately 2 mm to make them out of occlusion[1,8].

The prosthesis must be disinfected before using it in the mouth with any suitable disinfectant like 2% glutaraldehyde solution.

Routine minor adjustments are carried out and the prosthesis can be seated in position immediately after surgery. A surgical pack can be placed in the defect area before placement of the obturator if necessary.

CASE REPORT

We have treated 11 patients undergone maxillary partial or subtotal resection with the immediate surgical obturator in last 2 years (Table 1). Out of 11 patients, 6 had Armany's Class I defect, 4 had class Ⅱ defect (Figure 3) and 1 had Class Ⅳ defect[13]. All 11 patients were assessed at the interval of 1, 2, 6 and 12 mo follow up visits for evaluation of healing process and evaluated for patients' own satisfaction level and clinicians' perception level for bilateral facial symmetry on VAS. Patients' satisfaction level was recorded on VAS (with 0 indicating no satisfaction and 100 indicating complete satisfaction) and it ranges between 74% and 94% (with average of 87%) (Table 1). Four different prosthodontists have visually evaluated facial asym-

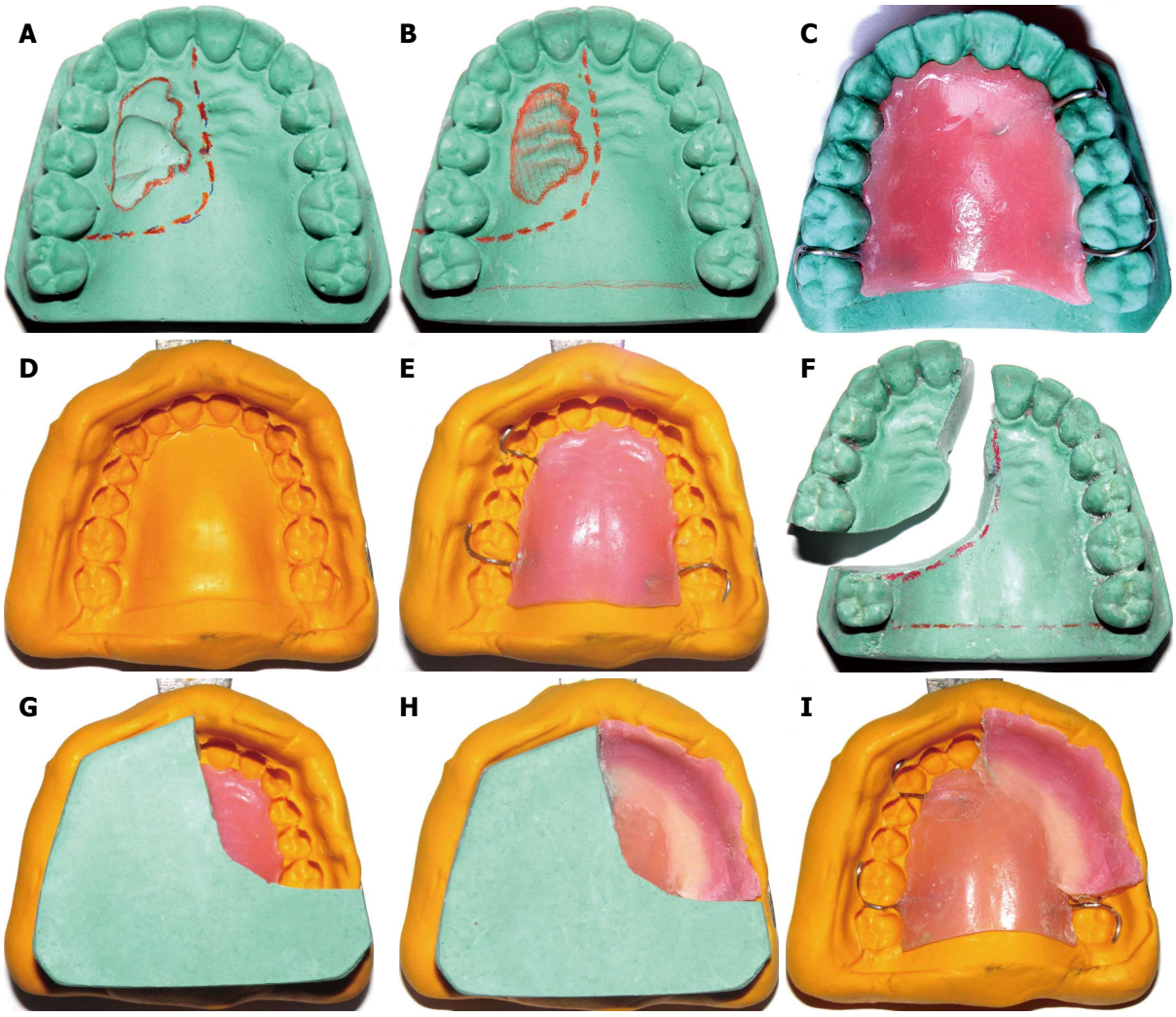

Figure 1 Technique to fabricate surgical obturator duplicating original tissue-form. A: Working cast; B: Modification of cast; C: Prosthetic base; D: Putty impression index; E: Prosthetic base transferred onto putty index; F: Maxillary cast sectioned; G: Reseating of putty index onto sectioned cast; H: Creation of the prosthetic teeth in tooth-colored and facial surface in pink-colored resin; I: Removal of cast from index.

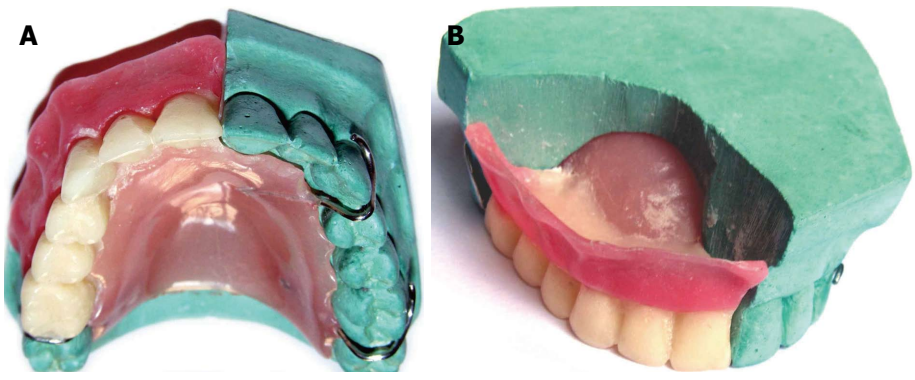

Figure 2 Completed obturator. A: Palatal view; B: Obturator in relation with remaining normal structures on cast.

metry of patients at 6 mo recall visit and indicated their visual perception for symmetry of the face on VAS (with 0 indicating completely asymmetric face and 100 indicating completely symmetric face). The average VAS scores of 4 clinicians' readings were calculated and presented in Table 1. Clinician perception VAS scores vary between 71% and 93% (with average of 84%).

DISCUSSION

Neglecting timely prosthodontic rehabilitation may lead to inappropriate facial contour which is difficult to correct[14-16].

Table 1 Details of the patients enrolled and visual analogue scale scores for patients' satisfaction level and clinicians' observation for facial asymmetry at 6 mo recall visits

Sr. No	Patient's surgical defect-type (Armany Classification)	Age (yr)	Sex (M/F)	Diagnosis	Post-surgical radiotherapy (Y/N)	Patient's satisfaction level (VAS at 6 mo)	Clinicians' observation for facial asymmetry (Average VAS scores of 4 clinicians at 6 mo)
1	Class Ⅱ	47	F	Squamous cell carcinoma of right maxilla	Y	86%	84%
2	Class Ⅰ	45	M	Adenocarcinoma of left maxillary sinus	Y	78%	86%
3	Class Ⅱ	50	F	Squamous cell carcinoma of maxillary sinus	Y	92%	80%
4	Class Ⅳ	63	M	Ameloblastoma of palate	N	93%	88%
5	Class Ⅰ	50	F	Squamous cell carcinoma of right maxilla	Y	88%	74%
6	Class Ⅰ	65	F	Squamous cell carcinoma of left maxilla	Y	94%	93%
7	Class Ⅰ	65	F	Squamous cell carcinoma of right maxilla	Y	83%	71%
8	Class Ⅱ	40	F	Squamous cell carcinoma of hard palate	Y	94%	93%
9	Class Ⅰ	36	M	Squamous cell carcinoma of Maxillary sinus	Y	81%	86%
10	Class Ⅰ	35	F	Squamous cell carcinoma of right maxilla	Y	74%	73%
11	Class Ⅱ	48	M	Ameloblastoma of left maxilla	N	94%	92%

M: Male; F: Female; VAS: Visual analogue scale.

The significance of immediate surgical obturators has been well documented. Immediate obturators resist tissue contracture of the soft tissues that are not supported by the underlying osseous structures. During healing phase, surgical wound is protected from external irritants, contaminants, food debris and trauma[17,18].

Advantages of adding flange and teeth

The borders of the defect are more prone to collapse due to lack of underlying support. Addition of teeth and labial/buccal flanges provide maximum support to the borders. Facial contours of the immediate obturator described in this article support overlying skin and skin grafts with optimum pressure providing their close adaptation to the cavity walls without getting contracted. After the operation, patients are able to swallow food more readily and to resume a normal diet at an earlier stage which leads to shorter recovery period[4]. Addition of teeth to the obturator allows mastication of semisolid food in initial phase and solid food several days later[4,14]. Speech is minimally altered and in many instances remains nearly unchanged[19,20]. Also the awareness of the surgical defect by the tongue is prevented and the patient remains unaware of the size of the defect giving the patient a positive psychological boost. By maintaining facial contour and aesthetics, patients are psychologically better equipped to face rehabilitation[21].

Purpose of replacing teeth immediately

Immediate restoration of the resected tissues by means the obturator that restores every missing portion of the tissues helps patients undergo unnoticed to the surgery. This gives patient positive psychological boost during the initial vulnerable period of healing. According to many authors, the posterior teeth should not be added to surgical obturator as they may exert unnecessary stress on the open wound and delay the healing process[3]. This technique describes replacement of dentition that would be missing followed by grinding occlusal contacts of posterior teeth (at least 2 mm) to position them out of occlusion. The facial surfaces must be kept intact to serve the purpose of facial soft tissue support as well as esthetics without disturbing the healing process. Anterior teeth should not be altered unless the incisal contacts hinder the healing tissues. The purpose of adding missing teeth (anteriors or posteriors) may prevent significant psychological trauma to the patient and helps to prevent scar contracture and subsequent disfigurement. The developed facial flange also helps to support the facial soft tissues which can maintain the patient's original facial esthetic appearance.

Catch at early healing stage

Contracture of the wound and scar formation leave very serious facial deformities after cancer surgeries[22]. Even slight depression in either side will also cause major deformity due to face value and patient's vulnerability towards esthetic appearance. If the radiotherapy follows the surgery the tissues become even more friable and are difficult to manage due to loss of natural laxity. Maximum wound contracture happens in initial stages within 4-6 wk. This is the period in which the losses of intraoral structures also cause difficulty in mastication and deglutition. Thus in initial stages of healing, patients may

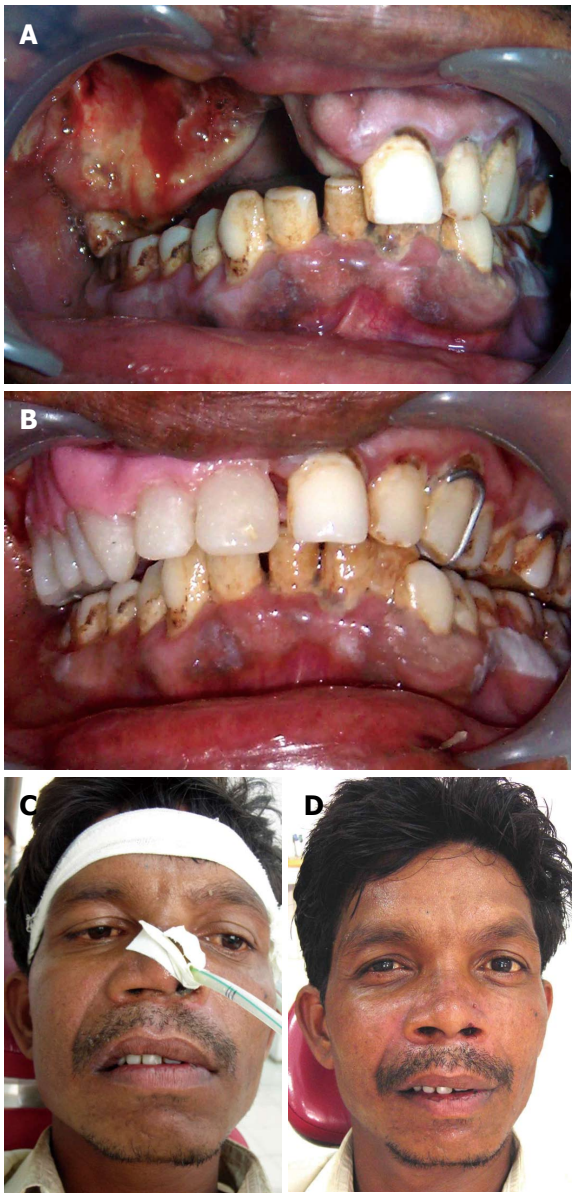

Figure 3 Post-treatment view immediate and after 6 mo. A: Immediate post-surgical view; B: Obturator in place; C: Immediate post treatment extraoral view; D: Six month post treatment extraoral view.

undergo severe psychological depression due to multiple problems. Immediate obturation can create a positive effect on the patients' psychology.

Works as Interim obturator

Interim obturators with teeth may be made using several methods, using a celluloid matrix[2], modifying a surgical obturator[7], using a denture duplicator[23], or using light[11,24] or heat-polymerized acrylic resin[25]. The obturator fabricated with this technique utilizes the PII of patient's original tissue form and duplicated mostly in heat and slightly in autopolymerizing acrylic resin.

Time and cost effective

Same surgical obturator can later (for 4-6 mo) serve as an interim obturator following modification of the tissue surfaces thus it saves time and cost.

Housing for placement of surgical pack

The space automatically formed between intaglio surfaces of facial flange and palatal plate can easily be utilized for placement of the surgical pack immediately after the surgery. Thus the obturator can provide supporting and stabilizing medium for the surgical pack.

Future dental implant planning

The same surgical obturator can be an effective tool for implant retained definitive fixed or removable prosthesis. Most of the acquired defects are surgically covered with thin mucosa which is not able to support denture bases. In such situations dental implants are indicated, leaving the vulnerable and/or non keratinized mucosa unloaded[26]. The use of dental implants is an alternative option to achieve better function and self confidence due to improved retention and stability[27]. Edentulous patients undergoing partial maxillectomy must be treated with implant supported ball and socket attachments to achieve retention to the surgical obturator. Same obturator can be used to prepare the diagnostic as well as surgical template for dental implant placement.

Limitations

As the teeth and facial flanges of the obturator are created in auto polymerizing acrylic resin, the free residual monomer may irritate the supporting tissues and hamper the healing process. The light polymerizing acrylic resin can be used alternatively to solve this problem provided the combinations of the light polymerizing acrylic resins and the methylmethacrylate based denture base resins were selected carefully to ensure sufficient bond strength[11,23,24,28].

Future scope

Future prospective clinical trials with large sample size should be promoted to treat cancer patients with better esthetics and function.

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CASE REPORT

Soft tissue aneurysmal bone cyst of the mandible: Report of a case

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Abstract

We report the case of a 17-year-old boy with a soft tissue aneurysmal bone cyst (STABC) located in the posterior aspect of the right mandible. Conventional radiography revealed no positive findings. On the computed tomography scan, the lesion appeared to have a non-uniform intralesional density. Magnetic resonance imaging revealed an abnormal soft tissue masses with cystic component in the superficial part of right mandibular body and angle with intact cortex. Following histopathological examination, fibro-histiocytic proliferation, blood-filled spaces and multinucleated giant cells were seen and the lesion was diagnosed as a STABC. The mass together with underlying bone and periosteum on its periphery was surgically resected under general anesthesia. Thirty-six months after surgery the patient was assessed at outpatient clinic and found no sign of recurrence This may be only the first reported case of the mandible in the English literature of this extremely rare benign tumor occurring in soft tissue.

Key words: Bone cysts; Aneurysmal; Mandible; Neoplasm; Soft tissue

Core tip: A case of soft tissue aneurysmal bone cyst (STABC) of the mandible is presented in a male teenager. STABC is a rare entity with histological and radiological features that are identical to those of aneurysmal bone cyst, except for that STABC is of extra osseous location. The differential diagnosis of STABC in this location, includes giant cell tumor of soft tissue and extra skeletal osteosarcoma making it quite a challenge in the process of diagnosis.

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INTRODUCTION

Aneurysmal bone cyst (ABC) is a benign cystic lesion of bone, composed of blood filled spaces separated by connective tissue septa containing fibroblasts, osteoclast-type giant cells and reactive woven bone[1]. Previously, ABC was believed to occur exclusively in bone[1,2], but in recent years a few cases of soft tissue ABC (STABC) have been reported[3,4]. The first cases of soft tissue ABC were reported by Salm *et al*[5]. These cases were categorized as "vascular cystic tumors of soft tissues". Recent literature review of well-documented cases shows that STABC is a recognized lesion and extremely rare[6]. To the best of our knowledge the reported cases of STABC have been located in thigh, cervical spine, shoulder and upper extremities[7,8] and STABCs in the jaws have not been reported in the English language literature.

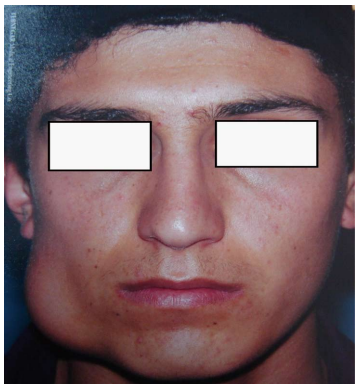

Figure 1 Clinical view. Facial asymmetry was apparent with, a firm, non fluctuant and non tender mass covered by normal skin on the right mandibular angle.

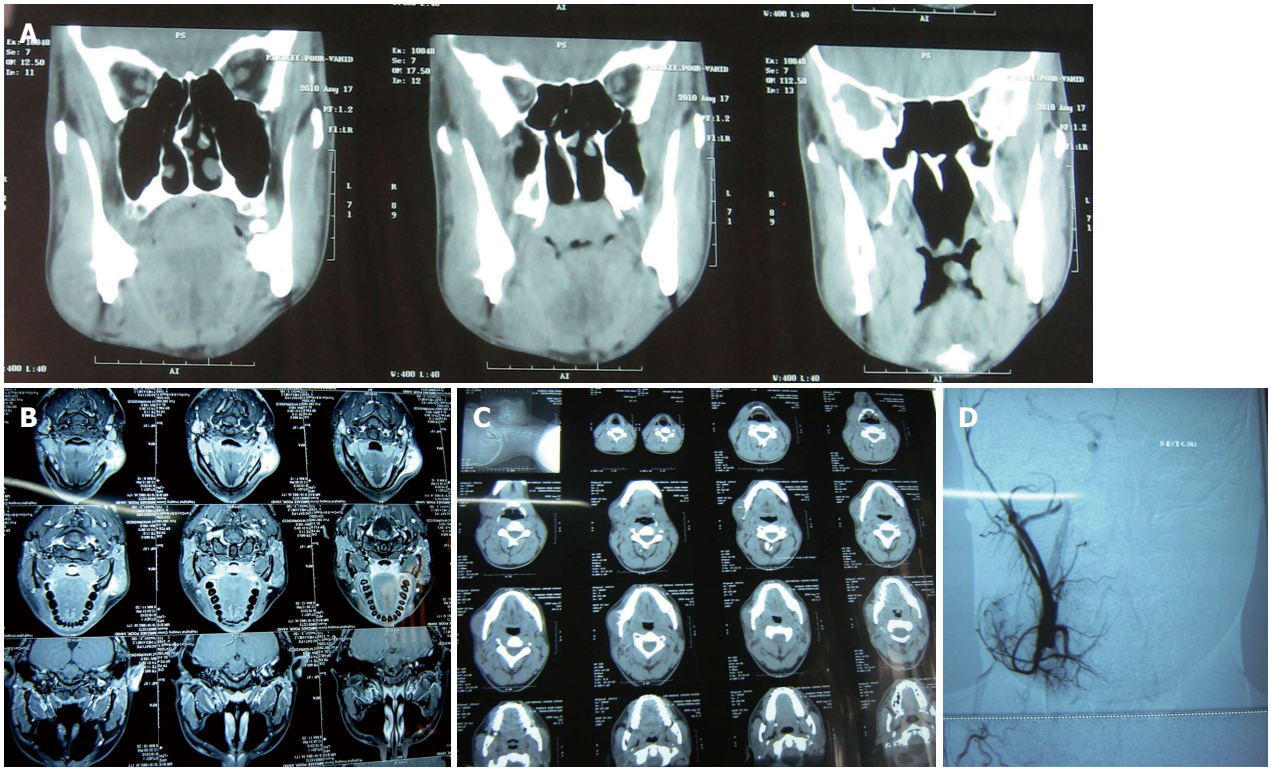

Figure 2 Computed tomography, magnetic resonance imaging and angiography. A: Coronal-axial computed tomography scan showed a lesion appeared to have a non-uniform intra lesional; B: Magnetic resonance imaging (MRI), T1 post contrast view demonstrated a well defined lesion with high signal intensity in the superficial part of right mandibular body and angle; C: MRI, T2 post contrast view demonstrated a well defined lesion with high signal intensity in the superficial part of right mandibular body and angle; D: Angiography of right carotid artery showed a lesion with only mild to moderate vascularity and ruled out arteriovenous malformation and hemangioma.

To increase our understanding of ABC arising in soft tissue, we report here a very rare case of soft tissue aneurysmal bone cyst of mandible. This case report was conducted in accordance with the principles of the Declaration of Helsinki.

CASE REPORT

In August 2009, a 17-year-old man was referred to a dentist complaining of a small swelling in his lower right angle of mandible beginning 4 mo ago. The patient was otherwise healthy, with no significant past medical history. He also did not have any previous history of trauma to head and neck region. The dentist suspected an inflammatory lesion and prescribed antibiotics. The patient continued the antibiotic use for one month but the size of the lesion did not change and began to increase. The patient was referred to Oral and Maxillofacial Surgery, Department of Buali Hospital in Tehran. On physical extra oral examination, facial asymmetry was apparent with, a firm, non fluctuant and non tender mass covered by normal skin on the right mandibular angle (Figure 1). On intra oral examination a firm swelling was found on the right mandibular angle without any fistula or suppuration. There were no positive findings in panoramic radiography. On magnetic resonance imaging (MRI), an abnormal soft tissue mass with cystic component in the superficial part of right mandibular body and angle with intact cortex (Figure 2A-C). In fine-needle aspiration, blood was detected and angiography was requested to rule out vascular lesions.

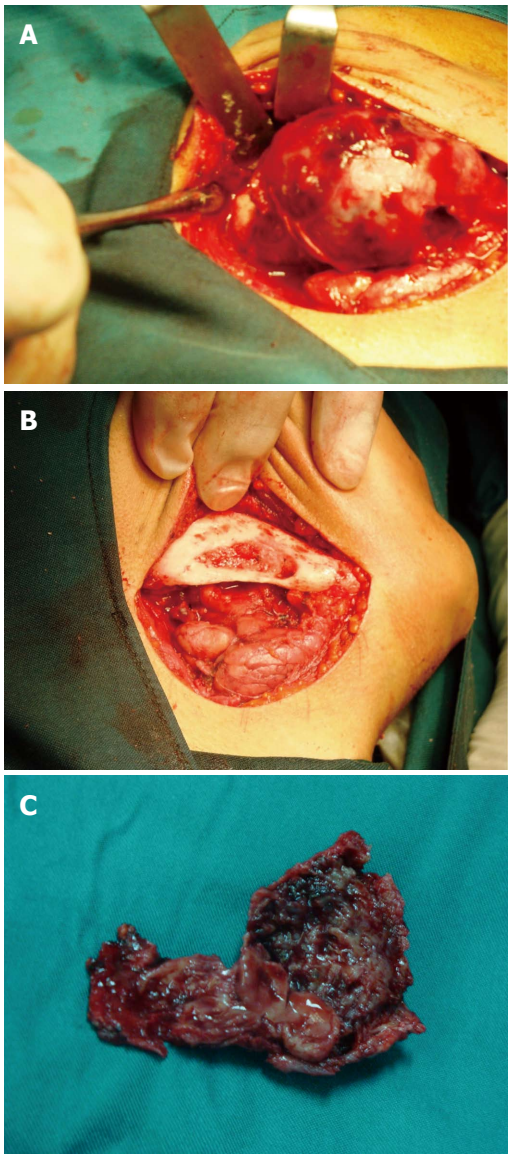

Figure 3 Clinical features at the surgery time. A: The mass was seen without involving adjacent bones; B: Periosteal reaction was seen in underlying bone; C: Gross view of excised lesion showed a solid lesion with eggshell-like rim of bone on its periphery and hemorrhagic cystic space.

The angiography report showed a lesion with only mild to moderate vascularity and ruled out arteriovenous malformation and hemangioma (Figure 2D). An incisional biopsy through an intraoral approach was performed under general anesthesia. The specimen consisted of 5 pieces of brownish-creamy fragmented tissues with rubbery consistency and solid on cut surface totally mesurring 3.0 cm × 2.0 cm × 0.4 cm, sent for histopathological examination. The microscopic evaluation showed spindle cell proliferation, many multi-nucleated giant cells, osteoid formation and pools of blood without any epithelium linings. Three weeks after the incisional biopsy, the patient was hospitalized and decortication of the lesion was surgically carried out under general anesthesia (Figure 3A and B). During the operation, frozen sections were requested and revealed no malignancy. Grossly, the mass was totally measured

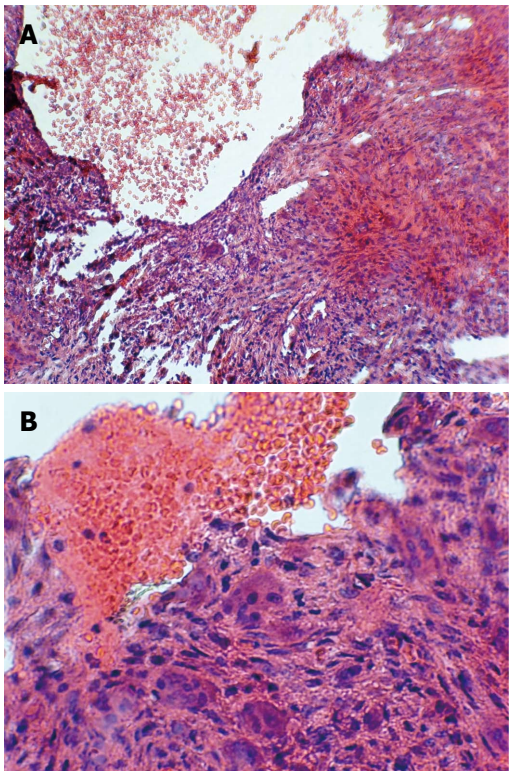

Figure 4 Pathological findings. A: Blood-filled space, fibro histiocytic stroma and multinucleated giant cells (HE × 100); B: Blood-filled space and multinucleated giant cells (HE × 400).

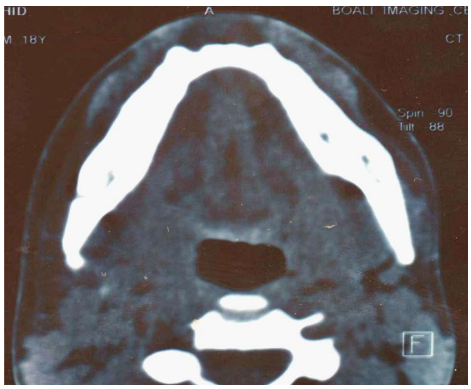

Figure 5 Coronal-axial computed tomography scan showed the patient is free of any lesion, 12 mo after surgery.

6.5 cm × 4.2 cm × 1.2 cm and showed a soft tissue lesion with hemorrhagic cystic spaces (Figure 3C).

Histopathological examination of the main lesion and the underlying bone and periosteum revealed fibro-histiocytic proliferation, blood-filled spaces and multinucleated giant cells (Figure 4). On reviewing all histopathological sections and paraclinical tests, the definitive diagnosis of STABC was confirmed. After 36 mo of follow up examination, the patient is well and free of any lesion (Figure 5).

DISCUSSION

STABC is a rare entity with histological and radiological

features that are identical to those of ABC, except for that STABC is of extra osseous location[4,5,7,8].

To the best of our knowledge the reported cases of STABC have been located in thigh, cervical spine, shoulder and upper extremities[3,8] and STABCs in the jaws have not been reported in the English language literature.

Our reported patient was male and a teenager, the age of the patient is in agreement with Nielsen *et al*[3] reporting five cases of soft tissue aneurysmal bone cyst in three females and two males who ranged from 8 to 37 years (median 28 years). Their reported cases arose in the soft tissues of upper extremities, tight and groin region as a rapidly growing mass. Clinical evaluation of our patient revealed rapidly growing non fluctuant and non tender mass on the right angle of mandible. Rapidly growing feature of ABC is a result of pathogenesis of this lesion and abnormal hemodynamics that leads to enlarging and hemorrhagic extravasation[3,6]. The maximal diameter of the lesion was 6 cm and macroscopically, the soft tissue mass was consisted of blood-filled cavities separated by septa of various thickness which is similar to the findings in other reported STABC[3,6,7].

Radiographically, the lesion had no positive findings in panoramic radiography. On the computed tomography scan, the lesion appeared to have a non-uniform intra lesional density.

MRI revealed an abnormal soft tissue mass with cystic component in the superficial part of right mandibular body and angle with intact cortex which is similar to the findings in other soft tissue ABC reports[7,8].

The changes seen in MRI appearance of aneurysmal cyst of soft tissue depends on its stage ranging from a primarily solid tumor to a predominantly multicystic lesion [9].

Histologically our reported case revealed fibro-histiocytic proliferation, blood-filled spaces and multinucleated giant cells, which is similar to other reports[3,4,6].

The differential diagnosis of STABC in this location, includes giant cell tumor of soft tissue and extra skeletal osteosarcoma[10].

Soft tissue giant cell tumor can be confused with STABC because of the presence of osteoclast- type giant cells in both lesions, but cystic change is the prominent view of STABC in histopathological evaluation[11,12].

Follow up information of our reported patient after 36 mo revealed no tumor recurrence, a similar finding compared to other reported cases[3,8]. On the contrary, local recurrence in soft tissue giant cell tumors are very common[12] and it may be considered as another factor differentiating STABC from soft tissue giant cell tumor.

Extraskeletal telangiectatic osteosarcomas, which are very rare, have gross features similar to those of STABC[10]. However, histologic examination of the STABC shows cells without cytologic atypia that are seen in extra skeletal telangectatic osteosarcoma[3,10].

The etiology of STABC is unclear. Several investigators have proposed trauma and vascular malformation as etiological factors[1,2]. However recent cytogenetic studies have provided evidence that STABC may be neoplastic in origin[13,14].

Follow-up showed that the patient has been free of any lesion 36 mo after the surgery, a good point to indicate that this lesion can be treated by simple excision and this treatment modality was in agreement with the report of Nielsen *et al*[3].

In conclusion, based on our experience STABC is an extremely rare type of benign soft tissue tumor especially in the head and neck area. Morphologically, it may be confused with a variety of soft tissue tumors. STABC infrequently recurs and complete excision is an appropriate treatment.

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