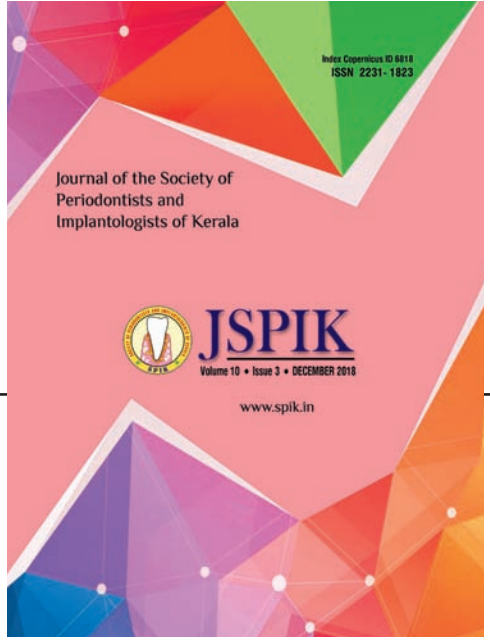
Index Copernicus ID 6818
ISSN 2231- 1823

Journal of the Society of Periodontists and Implantologists of Kerala

JSPIK

Volume 10 • Issue 3 • DECEMBER 2018

www.spik.in



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President's message

Greetings dear SPIK members……

Let me at the outset wish all members of SPIK fraternity a merry Christmas and a wonderful new year 2019. The current association year has been very encouraging as we had a lot of new faces coming and taking part in our activities. The Scientific programme on implants held at PMS Dental College witnessed great participation especially from PG students from across the state. The Programme was well appreciated by everyone. The organizing team headed by Vice President Dr Presanthila Janam and senior Professor Dr Seba Abraham needs special mention for the excellent manner in which they had conducted the event. The faculties were good and the hands on and problem solving sessions were very productive. The Second issue of JSPIK was released during the occasion and I do really appreciate the contribution to SPIK of Dr Plato Palathingal as editor.

This year, we are introducing the **first post graduate Gold medal examination** in Periodontics. The planning is in progress for the event and it is slated to be held in April/May 2019. This is envisioned to identify and promote the best young minds in Periodontology. The new year, we are all set to witness a sea change in the activities, as we gear up to organize the combined Annual conference and post graduate convention in February at Kochi. I really look forward to it. Eventhough we planned it a couple of years before we could not make it happen, but the organizing team headed by Dr Majo Ambookken and Dr Jayan Jacob are leaving no stones unturned to realize the dream this time around. As we approach towards the fag end of my tenure as President I acknowledge the support and contribution of each one of you in making this year fruitful to the fraternity. I urge all the senior members and teachers of our society to rope in more new members to strengthen the society further.

Looking forward to meet you all during the upcoming Annual Conference

Dr SeemaThampi

Thanking you,

Dr Seema Thampi
President, SPIK

Editorial

Warm Greetings to All of You.

Welcome to the 2nd issue- December of JSPIK. As now the curtains are down for the 43rd ISP National Conference at Chandigarh. I hope this JSPIK issue will reach your hands before New Year 2019. Upcoming SPIK Conference in Feb at Kochi with Scientific lectures will be very helpful for the postgraduates. SPIK helps to update our knowledge through these scientific sessions. We are also waiting to attend PG convention in March. JSPIK gives good opportunity to the post graduates as well as specialists in periodontics to write down and publish on their good clinical cases and original research articles. Please don't hesitate to contact me with your suggestions on JSPIK.

HAPPY NEW YEAR 2019

Dr Plato Palathingal
Editor
editorspik@gmail.com

Secretary's Message

As we approach the New Year, there are a lot of expectations and a lot of resolutions being made. Periodontology is witnessing great progress as a promoter and driving force of modern restorative dentistry. Implantology is the new momentum that propels modern dentistry and periodontology fuels the advancement. More and more specialists now understand the value of regenerative periodontics as they see alveolar ridges being sculpted and sound platforms being created to realise their oral rehabilitation concepts. Guided bone regeneration (GBR) is a boon to periodontology and we as periodontists should strive to benefit from the new found success in the three dimensional regeneration of alveolar bone. We now understand that it is possible to grow new bone and take the alveolar crest to new heights with our predictable techniques. We now see some light at the end of the tunnel and it is really encouraging.

It takes a lot of training and practice to master the skill of GBR. Let us include it in our wish list for the New Year. Let us all rededicate to try and learn a new skill in perio like GBR this year. I urge all the new kids on the block to identify the clinical skill that you want to add to your repertoire and make sincere efforts to learn that. If you have already learnt and never tried, try it soon and add to your kitty. Let the New Year make you alla better periodontist and better clinician.

SPIK as a specialty organization is committed to bring all the advancements happening in perio around the world to its members through training programmes and other scientific activities. So I appeal all the young professionals to join the party and get benefitted.

It gives me great pleasure as the new issue of JSIK is being brought out on time. It is being done meticulously with the determination and hard work of our young and exuberant editor Dr Plato. I take this opportunity to extend my sincere gratitude to my fellow office bearers including president Dr Seema Thampi, treasurer Dr Vivek Narayanan and scientific convenor Dr Arun Sadasivan for their commitment and contribution to SPIK. It indeed was a team effort and everyone deserves appreciation for that.

Wish you all a very happy new year

Perio thrills

Dr. Baiju R.M.
Secretary, SPIK

JSPIK

Co-relation of oral health status and intelligence quotient (IQ) levels among differently abled individuals: an observational study

Jose Paul[1], Johnson Prakash D'lima[2], Senny Thomas[2], Deepak Thomas[3], Binitta Paul Anand[3], Bini Varghese[4]

ABSTRACT

Aim: The aim of the study was to determine the oral health status and investigate the association of oral health status with IQ levels among differently abled individuals.

Materials and method: The study sample comprised of 100 differently abled individuals. Clinical assessment of oral hygiene was done using Oral Hygiene Index for specially abled individuals by James PMC and Gingival Index by Loe and Silness. Periodontal status was assessed by CPITN Index. IQ level was assessed using the Wechsler Intelligence scale.

Statistical Analysis: The relationship between IQ and parameters like oral hygiene status, brushing frequency and provision for help was analysed using KRUSKAL WALLIS ANOVA and the relationship between the same with GI and CPITN were assessed using one way ANOVA followed by TUKEYS POST HOC TEST.

Conclusion: The population of the present study had a fair oral hygiene and it was not statistically influenced by their IQ levels. This maybe be attributed to the provision of help that they were receiving. This highlights the need for an epidemiological survey followed by the implementation and evaluation of a long-range public dental health care plans

Key words: Oral Hygiene Status, Intelligence Quotient (IQ) Levels, Differently Abled, Periodontal Health

Introduction

The human race boasts of multitudes of diversity in terms of race, ethnicity, cultures and much more. In this complex assortment of humans, are a group of people whom the society has simply termed as 'the disabled'. Disability is described as an impairment that may be, developmental, cognitive, intellectual, physical, sensory, mental or some combination of these. It substantially affects a person's life activities and may be present from birth or occur later in life[1]. The term disabled has been losing its approval in recent times with several alternatives cropping up. One of these is finding more acceptance than others is - 'Differently Abled' which is term inclusive of all disabilities and offers an equal platform to those who fall under it.

As per the Census 2011, the differently-abled population in India is 26.8 million which accounts for a total percentage of 2.21%. There has been a marginal increase in the differently-abled population in India, with the figure rising from 21.9 million in 2001 to 26.8 million in 10 years. The total number of differently-abled people is over 18 million in the rural areas and just 8.1 million accounted for in the urban settings[2].

Differently abled individuals have been reported

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in literature to have more untreated caries, poorer oral hygiene and periodontal status with increased number of missing teeth[3]. They have physical, mental, sensory, behavioural, cognitive, emotional and chronic medical conditions which require health care beyond that is considered routine. This calls for an increased awareness, specialized knowledge, attention and accommodation for them[4].

These individuals may have potential motor, sensory and intellectual disabilities which limit their oral hygiene performance[5] and so are prone to poor oral health. They may also not understand and assume responsibility for or cooperate with preventive oral health practices[6]. Those that are very young, those with severe impairments, and those living in institutions are dependent on parents, siblings or

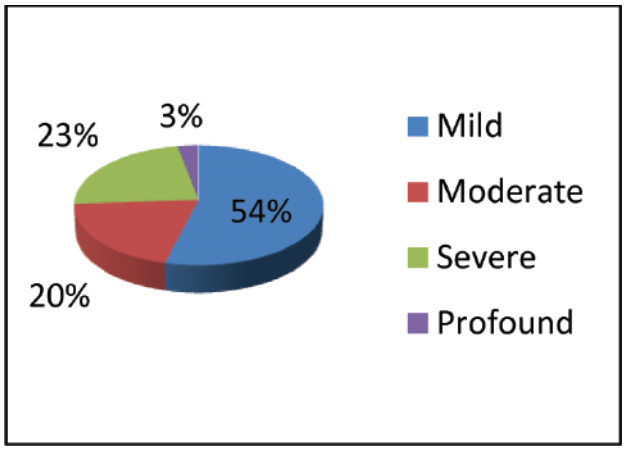

Fig 1: Degree of Intellectual Disability

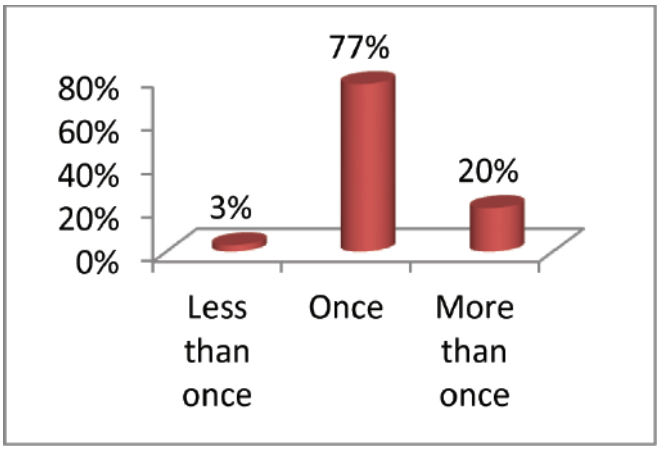

Fig 2: Frequency Of Brushing

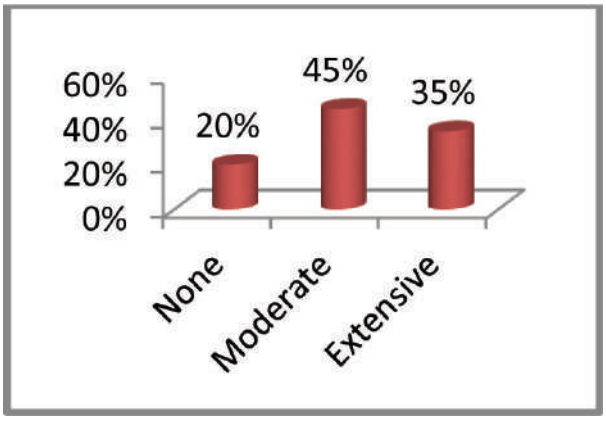

Fig 3: Assistance with Oral hygiene

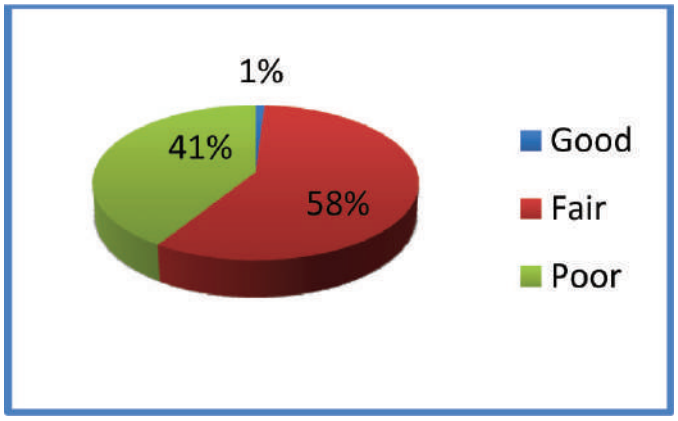

Fig 4: Oral Hygiene Status

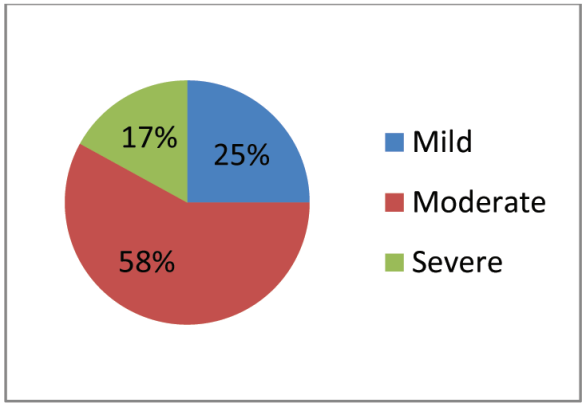

Fig 5: Gingival Index Score

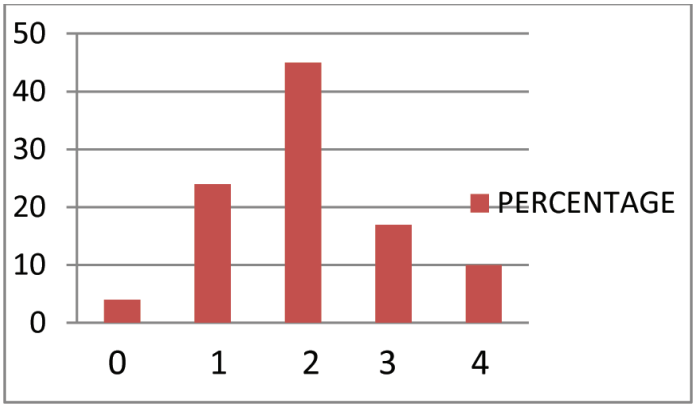

Fig 6: CPITN Scores

caregivers for general care including oral hygiene. Hence, signifying the importance of care givers in the lives of such individuals[7].

A higher prevalence and greater severity of periodontal disease than the general population is found in people with an intellectual disability (ID)[8,9]. The WHO has also developed an operational definition of ID, which focuses mostly on the functional elements of ID, based on the Intelligence Quotient levels and it has classified intellectual disability(ID) on the basis of severity.[10]

This study was carried out to determine the correlation of Oral Health Status and Intelligence Quotient Levels in a differently abled population taking into regard parameters like brushing habits, provision of help and clinical parameters like Oral Hygiene Index, Gingival Index and CPITN index, in order to provide information for future planning and intervention.

Objective

To determine the Oral Health Status of the differently abled population.

To investigate the co-relation of Oral Health Status and Intelligence Quotient (IQ) levels among them.

Materials and method

The study was conducted in a special school for differently abled located in Chalakudy, Kerala. The study sample included 100 differently abled individuals within an age group of 4-40 years, with varying degrees of mental retardation. Prior consent was obtained from the concerned authorities and study was approved by the Ethical Review Board. The following parameters were analysed:

Brushing Habits: The frequency of brushing, whether Nil, Once daily or Twice daily were noted.

Assistance For Oral Hygiene Measures: Most of these individuals were assisted by caretakers in their day to day activities including their oral hygiene practises. Hence, the degree of assistance was recorded as Nil, Moderate or Extensive.

Oral Hygiene Status: This was noted according to an index given by James et al[11] in 1960 for differently abled individuals and carried out using only visual examination. The index has three categories:

GOOD: The teeth are clean and there is no sign of food debris or materia alba.

POOR: The teeth are very dirty and there is considerable long standing food debris or materia alba.

FAIR: This class falls in between the two preceding ones and there is some evidence of debris, but not of the degree recognised as poor.

Gingival index (Loe and Silness 1963) and CPITN index were scored.

Intelligence Quotient (IQ): WESCHLER INTELLIGENCE SCALE (WISC) for children (0-16) and adults (WISA)>16 years of age was used to determine the IQ levels which were obtained from their medical records.

The population was classified into varying degrees of mental retardation based on the intelligence quotient levels according to the WHO classification of intellectual disability[10] as Mild intellectual disability (ICD-10 F70): IQ range 50 to 69

Moderate intellectual disability(ICD-10 F71): IQ between 35 and 49

Severe intellectual disability (ICD-10 F72): IQ between 20 and 34

Profound intellectual disability (ICD-10 F73): IQ less than 20.

Statistical analysis:

The relationship between Intelligence Quotient (IQ) score and parameters like oral hygiene status, brushing frequency and provision for help was analysed using KRUSKAL WALLIS ANOVA and the relationship between the same with GI and CPITN were assessed using one way ANOVA followed by TUKEYS POST HOC TEST.

Results:

The population size analysed was 100 which included 51 Females and 49 males. The age group included individuals from the age of 4 to 40 years with a mean average age of 21.97. According to WHO classification for intellectual disability the population was divided on the basis of their IQ levels and it was found that around 54% had mild, 20% had moderate, 23% had severe and around 3% had profound intellectual disability (Fig 1). The

tooth brushing frequency was noted with around 77% of the population brushing at least once daily and around 20% of the population brushing twice daily. Only 3% individuals did not brush even once per day (Fig 2). When considering the provision of help they were provided with oral hygiene measures, it was found that around 45% acquired moderate assistance and 35% individuals were provided with extensive assistance. There was another 20% who did not need any supervision with oral hygiene measures (Fig 3). The oral hygiene status as scored according to the index given for differently abled individuals it was found that an average of 58 % had a fair oral hygiene status and around 41% had a poor oral hygiene status. Only 1% of the population was found to have good oral hygiene status (Fig 4).

The Gingival Index scores indicated a majority of the population that is around 58% had moderate gingivitis and 25% had mild gingivitis (Fig 5). The CPITN index scores were found to have a maximum score of 2 for around 45% of the population (Fig 6). On statistical analysis it was found that there was no significant relation between the oral hygiene status and the IQ levels of this population ($p>0.05$) and the overall oral hygiene status of this population was fair.

Discussion

One of the detrimental factors that can have dramatic effects on an individual's quality of life is poor oral health. In fact, it can affect different aspects of life including eating, speech impediments, pain, sleep disturbances etc. leading to missed days of work or school and decreased self-esteem.[12,13]

As noted in various studies the increased prevalence of oral health problems among individuals with Mental Retardation may be related to their oral health habits since oral health is dependent on oral hygiene[14]. Among individuals with intellectual disability (ID), those with moderate or severe ID have been found to brush their teeth more regularly than those with mild ID[15]. The same group, however, often have impaired physical coordination and cognitive sequencing skills that limit independence in task completion[16]. Consequently, they generally need assistance from caregivers to complete oral hygiene tasks. This is in congruence with the present study which found that more than 2/3rd of the study population had assistance with oral hygiene measures with 45% receiving moderate and 35% receiving extensive assistance with brushing habits and maintenance of oral hygiene. The overall oral hygiene status of this population was found to be fair (58%) and the CPITN INDEX scores obtained a maximum of score 2 which indicates a treatment need of improving the personal oral hygiene and professional scaling. This could be attributed to the level of assistance provided by the care takers. This brings to light the importance of the role these caregivers play in the overall wellbeing and specifically the oral hygiene needs of such a population. It also highlights the importance of instruction and reinforcement of daily oral hygiene among individuals with intellectual disability.

On statistical analysis it was found that there was no significant relation between the oral hygiene status and the IQ levels of this population ($p>0.05$) and the overall oral hygiene status of this population was fair. These findings contradict the results found in similar studies conducted to analyse the correlation between these two parameters.

In a study that was carried out on 252 children of 10-15 years old, in order to assess the relation between IQ of a child with dental caries and gingival disease, it was found to have a significant difference among different intelligence groups and the prevalence of moderate gingivitis ($P<0.001$). But no significant association was found between level of intelligence and caries prevalence.[17]

In another study conducted in Japan, 241 mentally retarded persons, institutionalized in three private welfare facilities in Oita Prefecture, were assessed for dental status and tooth brushing ability. The results showed that both low tooth brushing ability and low intelligence quotient were associated with their poor dental status[18].

The oral health status with supervised tooth brushing was assessed for 60 children with physical and mental disabilities from a special need school in India. The effect of supervised tooth brushing and changes in the plaque and gingival index in mentally challenged children were statistically insignificant but significant improvement was observed in the group with autistic children.[19]

It has been noted that inadequate knowledge is the major factor preventing caregivers from

inculcating a congenial oral health behaviour among differently abled individuals.[20] Therefore, Oral Health related educational programs specifically aimed at promoting caregivers' behaviour and attitude must be implemented. Along with supervised brushing and oral hygiene care, these individuals with special needs require regular dental visits and use of electronic brushes may be encouraged. The Dental Professionals may provide comprehensive school-based initiatives and workshops including oral health education to help children develop skills. They may use fluoride supplements and sealants along with dietary and nutrition counselling to promote oral health.

Also, similar studies need to be conducted on a larger population and at a larger scale with analysis of more important parameters, that affect not only the oral hygiene needs of people with special needs but also their overall wellbeing.

Conclusion

Everybody has ability and everybody matters, it's all about acknowledging it. The population of the present study had a fair oral hygiene which maybe be attributed to the provision of help they were receiving. Hence the need of the hour is an epidemiological survey followed by the implementation and evaluation of a long-range public dental health care plan for such individuals.

Acknowledgement

This study was made possible due to the support and guidance provided by Dr. Jose Paul, HOD & Professor, Dept of Periodontics, Annoor Dental College & Hospital, Muvattupuzha. Also we would like to acknowledge Sr. Anjo, Principal, Madonna Special School, Pota, Chalakudy along with all the staff who assisted in the smooth completion of this study.

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JSPIK

Evaluation of relationship between sleep deprivation and periodontitis: a pilot study

Achu Jerard[1], Jose Paul[2], Johnson Prakash D'Lima[3], Senny Thomas[3], Deepak Thomas[4], Binitta Paul[4]

ABSTRACT

Background: Lack of sleep presents a highly negative impact on the systemic health by causing a state of increased inflammation and deregulation of immunity resulting an infectious threat and increased potential for the incidence as well as progression of systemic diseases. Literature evidences manifesting this effect of sleep deprivation on systemic health pose a question of its similar impact on periodontal diseases as well,especially periodontitis whose underlying pathology involves a generalized state of inflammation and infection of tooth supporting structures.

Aim: The aim of the study was to determine the relationship between sleep deprivation and incidence and progression of periodontitis.

Materials and methods: A total of 60 systemically healthy subjects within the age group of 18 to 70 years were examined and categorized into three groups: Clinically healthy, Gingivitis and Periodontitis, each with 20 number of participants. The Gingival index and Probing Pocket depth were scored and sleep quality of each of the subject was assessed by administering them with PSQI questionnaire.

Results and Conclusion: The study brought out the results that shows a clear independent association between sleep duration and periodontal disease by demonstrating highest PSQI value associated with the group with moderate or severe periodontitis when compared with remaining two groups.

Key words:- Sleep, Periodontitis, PSQI, Systemic inflammation

Introduction:

Sleep isn't sleep anymore!! Instead is a door to a "healthy body with a healthy mind" with efficiently functioning bodily systems.

Sleep is classically defined as a cyclic, temporary, and physiologic loss of consciousness that is readily, promptly, and completely reversed with appropriate stimuli.[1]

On an average, human beings sleep for 8-9 hours a day thereby spending about one-third of their lives asleep[2], yet most individuals know very little about sleep. Although its role in body physiology, mechanisms and functions are still to be exemplified, there has been no doubt regarding its essentiality. However, sleep deprivation has emerged as a universal phenomenon due to the shift into a 24/7 lifestyle with high incidence of its consecutive pathologies, and whose primary etiology often goes extraneous and undiagnosed.

With growing evidences, Sleep! Which is referred as "body's rest cycle" is found to be essential for a person's health and wellbeing. Though in

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vicinity, it is regarded as a period of rest for body and mind, numerous physiologic activities related to cardiovascular system, respiratory system, muscular and endocrine systems are undertaken during the period, the absence of which may lead to serious biologic consequences. It has been proved to have a prime role in the adept functioning of the entire physiologic system in a human body. Short sleep duration has been independently linked to several diseases, particularly to diabetes mellitus, metabolic syndrome, hypertension, stroke and coronary artery diseases.[3-7]

Although the correct underlying mechanism by which lack of sleep is related to these pathologies is still to be resolved, the increased systemic inflammatory status and oxidative stress are evidently associated with the causal pathway of both the conditions, linking them up[4,8]. Recently, also the association between short sleep duration with the risk of systemic infections has been of focus, which is best illustrated by the experimental evidence of host immunity impairement.[9] These speculations compelled the researchers to further investigate any possible association between sleep duration and periodontitis, which is a chronic inflammatory disease.

Periodontitis is defined as the infectious and inflammatory disease of the periodontium that involves the destruction of the supporting structures of the teeth including the periodontal ligament, bone and gingival tissues, ultimately leading to tooth loss. It is widely regarded as the second most common dental disease in the world with a prevalence range of 30-50 % in the population of U.S and in India,which is the second most populated country that accounts for around 17.5% of the total world population[10], its prevalence rate was found to be 89.6% in the middle age group of 35-44 years as per the National Oral Health Survey and Fluoride Mapping (2002-2003), conducted by Dental Council of India, New Delhi in the year 2004.[11]

Periodontal disease is multifactorial in nature with the destruction involved in it as a consequence of the interaction of genetic, environmental, host, and microbial factors.[12] A "periodontal war" is initiated between the host tissues and the invading microbes that induces a complex cascade of inflammatory and immune responses, resulting in progression of tissue destruction.[13] Through various longitudinal studies, the vital role of numerous other risk factors like diabetes mellitus, smoking in the furtherance of periodontal diseases with periodontitis in specific has been established. The underlying mechanism that consociate these factors to periodontitis are seemed to be the over-all decrease in immunity and an increase in systemic inflammation with an upturn in inflammatory markers, which are also the classical features associated with sleep deprivation.[4,10,14,15,16]

Determination of the impact of "lack of sleep on periodontal health" is an evolving and novel area of interest in the field of dentistry as well as Periodontology in specific, with very limited number of studies on this regard.

The aim of the present study hence, is to evaluate and establish if there exist a relationship between sleep deprivation and periodontitis.

Materials and methods

Patient screening was carried out from the patients visiting the department of Periodontology and Implantology, Annoor Dental College and Hospital, Muvattupuzha (Kerala). After recording a detailed family history (which included information on patient's age, gender, socioeconomic status,occupation and lifestyle), medical and dental history, a total of 60 subjects were selected and had been categorized into 3 different groups,each of it with 20 subjects each.

Pregnant ladies, lactating mothers and Subjects,with less than a number of twenty teeth, who suffer from any systemic diseases, who had received any periodontal treatment in 6 months before study or those who had history of medication (antibiotics or anti-inflammatory drugs) in 3 months before commencement of the study were excluded from the study as these factors themselves would affect the sleep quality as well as periodontal status of the person.

A meticulous periodontal examination was carried out for all the subjects included in the study, where the assessment of periodontal status was made by scoring gingival index (GI) (Loe, 1963) and by measuring pocket probing depth and clinical attachment loss, by probing from the gingival margin

to the bottom of the periodontal sulcus or pocket at six sites of each tooth, using William's periodontal probe. The examination of all the study subjects were carried out by a single examiner in order to eliminate any possible bias.

Subjects were then grouped into following three groups based on the criteria below, followed by administering each of them with a standard

Name_____ Date_____

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1 _____
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2 _____
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3)	C3 _____
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=1, 65%-74%=2, <65%=3	C4 _____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5 _____
Component 6	#6 Score	C6 _____
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7 _____

Add the seven component scores together _____ *Global PSQI* _____

A total score of "5" or greater is indicative of poor sleep quality.
If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

Fig I PSQI questionnaire

questionnaire to assess their sleep quality.

• Group I – Healthy: GI score: 0, PPD≤3 mm

• Group II – Gingivitis: GI score ≥ 1, PPD ≤ 3 mm

• Group III – Moderate to severe generalized chronic periodontitis: Generalized - PPD ≥ 3 in ≥30% of sites; moderate-severe periodontitis - PPD ≥ 6 mm

The Pittsburgh Sleep Quality Index (PSQI):

The Pittsburgh Sleep Quality Index is a self - rated questionnaire which assesses sleep quality and disturbances over a one – month time interval, making it an effective tool to appraise ones sleeping pattern in an older adult.

It comprises of 19 individual self-rated questions and five separate questions rated by bed partner or room- mate, if one is available. These 19 individual items generate 7 component scores each of which has a score ranging from 0-3, where Score 0 indicates no difficulty in sleeping whereas Score 3 indicates severe difficulty. The sum of scores of these seven components yield one "global score" whose value lies within a range of 0-21 where score 21 indicates severe difficulties in all areas. A global sum of 5 or greater indicated poor sleep quality. Higher PSQI scores represented worse sleep quality.[17]

Enabling to easily differentiate "poor sleepers" from "good sleepers", it proves to be a simple, reliable method of assessing one's sleep that gives out a valid and standardized measurement of its quality.

A modified version of the original PSQI questionnaire (which included the first 9 items only) was used for the purpose of the study. (Fig I).

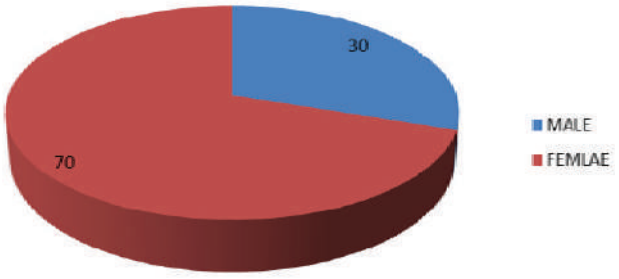


Fig II: Gender wise distribution of the participants

		N	Mean	Std. Deviation
G.I	1	20	.565	.2498
	2	20	1.495	.3103
	3	20	2.105	.3120
	Total	60	1.388	.7001
PPD	1	20	2.95	.224
	2	20	3.70	.657
	3	20	6.65	1.954
	Total	60	4.43	1.995
psqi2	1	20	1.25	.444
	2	20	1.35	.489
	3	20	1.85	.366
	Total	60	1.48	.504

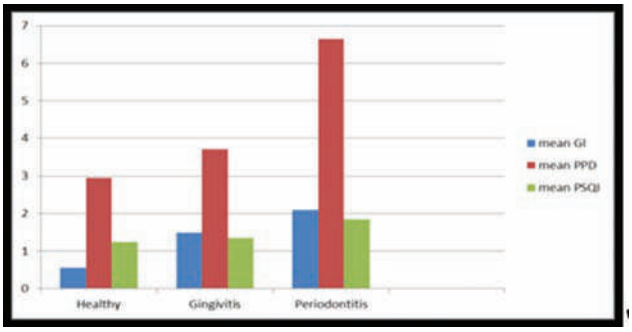
Table I: The mean with standard deviation calculated for GI,PPD and PSQI for the three groups 1, 2 and 3

	GROUP 1	GROUP 2	GROUP 3	ANOVA	TUKEY's POST HOC
GINGIVAL INDEX	0.56 ± 0.25	1.50 ± 0.31	2.10 ± 0.31	F = 140.95 p value = 0.000	3>2>1 HS
PROBING POCKET DEPTH	2.95 ± 0.22	3.70 ± 0.66	6.65 ± 1.95	F = 53.38 p value = 0.000	3>2>1 HS

Table II: Comparison of gingival index and probing depth scores expressed as mean with standard deviation among the three groups.

	1	2	KRUSKAL WALLIS ANOVA
GROUP 1	15	5	F = 16.28 p = <0.001 HS
GROUP 2	13	7	
GROUP 3	3	17	

Table III: Comparison of PSQI scores among the three groups



Graph I: The comparison of the mean values of GI,PPD and PSQI

Statistical analysis:

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 22.0. Descriptive data were presented as mean and standard deviation. ANOVA test was used for comparison between means of groups and to determine the significance of each parameter under study. Tukey's Post Hoc test was used for intergroup comparison of the scores of Gingival Index and Probing pocket depth. Kruskal Wallis ANOVA test was used for the comparison of PSQI scores among the three groups.

Results:

A total of 60 subjects who are systematically healthy and fall into the age group of 18–70 years were assessed, regardless of the gender to explore if there exist a relationship between routine inadequate sleep and chronic periodontal disease and following results were drawn out.

The GI, PPD and PSQI were expressed in the form of "mean with standard deviation" and was calculated for the three groups - Groups 1, 2 and 3.

For the three groups, the mean of GI, PPD and PSQI were revealed to be (0.56+/-0.25,1.50+/-0.31, 2.10+/-0.31), (2.95+/-0.22, 3.70+/-0.66, 6.65+/-1.95), (1.25+/-0.44,1.35+/ 0.48, 1.85+/-0.366) respectively (Table I)

Tukey's Post Hoc test was used for intergroup comparison of the scores of Gingival Index and Probing pocket depth (Table II) and KRUSKALWALLIS ANOVA test for the comparison of PSQI scores among the three groups (Table III).

The results were shown to be highly significant (HS) with the mean value of GI AND PPD highest for Group 3 followed by Group 2 and then Group 1TABLE II with the mean PSQI highest for the periodontitis group.

Intergroup comparison of PSQI scores satiated significant difference in its value amongst all three groups and the results showed no significant difference among the genders with respect to GI, PPD or PSQI.

Discussion:

Our study was conducted to potentiate the possibility of an association between sleep and periodontitis that would pin sleep deprivation as a risk factor for periodontitis. The present clinical investigation throws light on the impact of lack of sleep on oral as well as periodontal health with its substantiating results. In our study, the Pittsburgh Sleep Quality Index (PSQI) was used to assess the sleep quality and to categorize the subjects from good to bad/poor sleepers. Numerous studies using the PSQI in a variety of older adult population internationally have supported high validity and reliability.[17]

In this study, a positive correlation of PSQI with GI and PPD was observed. It was seen that the mean PSQI is highest for Group 3 followed by group 2 and lowest for group 1, which clearly marks out that the sleep quality of individuals with moderate or severe periodontitis seemingly is worse compared to those with gingivitis or healthy periodontium. These findings therefore affirm our hypothesis that there is a close connection between sleep duration and periodontitis and hence support the existing literature that manifest the underlying mechanism by which sleep and infectious or inflammatory diseases are hooked up.

Snoring and sleep apnea (apnea means cessation of breathing) are points along a spectrum that extends from benign or simple snoring with no sleep disturbance to obstructive sleep apnea (OSA) with excessive daytime sleepiness and the physiologic consequences of recurrent asphyxia.[18]

There are numerous studies that are conducted to associate sleep and related disorders with various entities like systemic inflammation, oxidative stress levels, hence their effect on systemic diseases.[8,13,14,19,20,24]

Oxidative stress and inflammatory reaction due to OSA have seldom been studied and studies have demonstrated a positive correlation of sleep apnea with oxidative stress and inflammatory biomarkers including C-reactive protein (CRP), interleukin 6 (IL-6), total antioxidant capacity (TAC), and urinary 8-hydroxy-2-deoxyguanosine.[8]

In a study conducted with an aim to determine how sleep deprivation acutely affects inflammatory markers, demonstrated that one night of total sleep deprivation in healthy young participants during strictly controlled constant routine conditions of bed rest, inactivity,dim light, and hourly nutrition

intake, significantly altered circulating levels of pro- and anti-inflammatorycytokines and cell adhesion molecules. A significant increase in sE-selectin and sICAM-1 levels, IL-1b and IL-1 & 6 levels were noted, indicative of an increase in the pro inflammatory and anti-inflammatory response respectively.[18] Sleep deprivation hence forth seems to exacerbate the inflammatory status and change the disease course, delaying healing and recovery.[19]

Several other studies have examined the effect of total sleep deprivation on cortisol, with many reporting an increase in its level.[20, 21]

Sleep quality is considered to have a critical role in prediction of immunity too. Lack of sleep seemed to reduce immunity, thereby increasing the susceptibility to numerous diseases. Insufficient sleep has shown to alter the immune responses by up regulating CD14, and bringing out variations in CD4 and CD8, henceforth increasing the susceptibility to infectious diseases.[14]

Apart from its effects on systemic inflammation and host immunity, it also seems to decrease the immunologic protection offered by standard vaccines.[22]

Though mechanisms and mediators of these associations are to be further investigated,these findings should help raise awareness in the public health community about the clear connection between sleep and health.

Periodontitis is characterized by a generalized state of inflammation of tooth-supporting structures that ultimately results in edentulism, posing a great negative impact on individuals' quality of life. Hence the global epidemiological data suggests periodontal disease to be one of a major burden on oral diseases.[23] To reduce this burden it is necessary to know the true prevalence of and etiological factors related to the disease according to which proper initiatives can be formulated.

Evidences advocating the increase in pro-inflammatory and anti-inflammatory markers like ICAM, E selectins and IL1, 6,17, TNF –alpha, dysregulation in antioxidant capacity as well as immunity as a result of sleep deprivation suggests a biological plausibility for it to be a factor that increases the likelihood of occurrence and progression of periodontitis.

A marked shift in the presence of particular oral and periodontally relevant microorganisms like A. actinomycetemcomitans (A.actinom), A. naeslundi, F. nucleatum, P. gingivalis, P. intermedia, P. micra, S oralis, S. mutans, T. denticola, T. forsythus, a in subgingival plaque could be detected in the sulcus and plaque samples in patients with obstructive sleep apnea which further affirms the result of our study.[24]

Sleep deprivation has also shown to affect the health as well as work performance, cognition of individuals that might influence the quality of personal and hygienic care that they themselves can render.[25] This can also influence their oral hygiene practices affecting the health and status of periodontium in a negative manner.

Though the sampling procedures and the data collection methods in the study allowed the minimization of the possibilities to have selection bias or information bias, the age and gender distribution and the less sample size can act as confounding factors which reduce the strength of the study.

Age seemed to be the key confounding factor in our study, since Group 3 which showed highest PSQI score included mostly of the sample people in an age group of range 40-65 years.

The occupational factors like work-time and stress also have shown to confound the association in an important manner, since all of these factors including age seemed to have a significant effect on the prevalence and causation of periodontitis even otherwise.[26,27,28]

There is no much previous literature or studies directly relating sleep duration and periodontitis to compare our results. However, recently, a paradoxical association between sleep duration and periodontitis has been studied and has shown that the prevalence of periodontitis increased with increase in sleep duration.[29]

Besides,due to the multifactorial nature of both periodontitis as well as sleep deprivation, drawing out a definitive line of independent relationship between the two is found to be a challenging task.

Conclusion:

Within the limits, the present study elicited a strong, independent association between sleep and periodontal diseases, possibly making it another important and commonly encountered risk factor in causation of periodontitis.

However, new studies with apparently larger sample size with equal proportion of male and female components, preferably with a shorter range of age group are called for, in order to draw better results with no shortcomings and thus to expand our understanding on this unique, highly interesting field of research to enlighten the world with the need for adequate sleep and better lifestyle practices.

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MicroRNAs – A novel approach to periodontal diagnosis

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ABSTRACT

MicroRNAs (miRNAs) are short, noncoding RNAs that act as key regulators of diverse biological processes by mediating translational repression or mRNA degradation of target genes. They are critical regulators of the host immune and inflammatory response against bacterial pathogens. In the periodontium, miRNAs play key roles in development and periodontal homeostasis and during the loss of periodontal tissue integrity as a result of periodontal disease. In this review, we are focusing on the potential role of microRNAs in periodontal disease diagnosis, and the role played by them in the disease pathogenesis. New therapeutic strategies represented by miRNA-based therapeutic approaches in periodontal disease could become a starting point for future development of novel therapeutic tools.

Keywords: MicroRNAs, Biomarkers, Periodontal Diseases, Diagnosis

Introduction

Lee et al.[1] in 1993, described that a small non-coding RNA in Caenorhabditis elegans was able to regulate the expression and function of another protein-coding mRNA. The discovery of microRNAs (miRNAs or miRs) had a profound impact on the understanding of many gene regulation processes in the following years. Since they were first discovered, the physiological relevance of miRNAs in regulating plant and animal gene expression has been established.

The primary repository for miRNA sequences and annotations, miRBase (www.mirbase.org), debuted in 2006 with just 218 miRNA loci.[2] Since then, novel high-throughput sequencing techniques applied to miRNA analysis have allowed the discovery of more than 38000 mature miRNAs (miRBase release 22, 2018). MiRNAs participate in the post-transcriptional regulation of gene expression in almost all key cellular processes,[3] such as regulation of cell proliferation, differentiation, angiogenesis, migration, and apoptosis.

Significant evidence has accumulated in the last few years, showing a fundamental role of miRNAs in the development of many diseases.[4] In particular, in cancer, aberrations in miRNA expression levels have been linked to the onset and progression of various types of cancer.[5]

Biogenesis of microRNAs

miRNAs are small, evolutionarily conserved, non-coding RNAs that are approximately 18-25 nucleotides in length and constitute the dominating class of small RNAs in most somatic tissues. Although many aspects of the miRNA biogenesis pathway and repressive mechanisms are still obscure, the key processes have been fully characterized.[6]

The mode of action of these regulators is through imperfect complementary binding to the 30 untranslated regions (30 UTR) of target mRNAs.[7,8]

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Typically, a single miRNA has the potential to simultaneously control the translation of hundreds of genes.[9, 10]

The process by which miRNAs are formed is called miRNA biogenesis. The biogenesis of miRNAs begins with the production of mRNA-like polyadenylated primary transcripts (pri-miRNA)by RNA polymerase II. Subsequent miRNA maturation requires two RNase III proteins, Drosha and Dicer. These two proteins may collaborate in the stepwise processing of miRNAs, and also have key roles in the process of miRNA-mediated gene regulation. The large pri-miRNA is then cleaved by the RNase III enzyme Drosha and coupled with the microprocessor complex subunit DGCR8 to produce pre-miRNA. Pre-miRNAs range from 70 to 90 nucleotides in length and contain a stem loop structure for their transport to the cell cytoplasm by Exportin-5. Once in the cytoplasm, this hairpin structure is cropped off by the RNase III enzyme, Dicer, producing the double-stranded miRNA:miRNA duplex. The mature single-stranded miRNAsare eventually incorporated into a ribonucleoprotein complex, the RNA-induced silencing complex (RISC). It is in this formation that RISC targets complementary mRNA sequences and exerts its cellular effects, via transcriptional cleavage or transcriptional repression (Fig. 1).[11, 12]

Significance of microRNAs in periodontal pathogenesis

During the last decade, miRNAs have emerged as critical regulators of the immune response based on their ability to interfere with the post-transcriptional expression of multiple target genes. During immune and inflammatory responses, miRNAs target inflammatory regulators and affect the magnitude of the inflammatory response. miRNAs are involved in the response against pathogens of bacterial, viral, fungal and parasitic origin. Studies have shown that miRNAs are dysregulated in periodontal disease. In periodontal disease, miRNAs exert control over all aspects of innate and adaptive immunity, including the functions of neutrophils, macrophages, dendritic cells and T and B cells. miRNA species play an inherent transcriptionally important function as specific alternative genetic inhibitory transcriptional endpoints of signaling cascades like Toll-like receptors (TLRs), nuclear factor kappaB (NF-$\varkappa$B), and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways, which play significant roles in periodontal pathogenesis.[13]

MicroRNAs as biomarkers

Gene expression profiling studies have demonstrated alterations in miRNA expression in a wide range of human diseases. In many cases, functional studies have linked miRNA dysregulation as a causal factor in disease progression. Alterations in miRNA expression have now been demonstrated in many cancer types. Early studies were performed using microarray, real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bead-based hybridization (Luminex) platforms, while more recent studies have used Next Generation Sequencing (NGS)-based profiling. Even though, real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR or qPCR) is often considered a "gold standard" in the detection and quantitation of gene expression, the rapid increase in number of miRNAs renders qPCR inefficient on a genomic scale, and it is probably better used as a validation rather than a discovery tool.[14-20]

miRNAs have been proposed as promising biomarkers of various diseases because they can be readily detected in tissue samples (non-circulating miRNAs) and are also stably found in body fluids

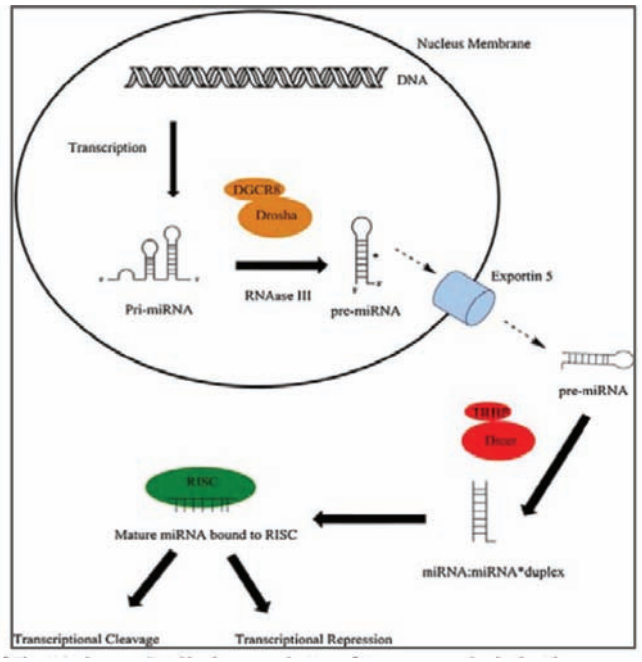

Figure 1: Steps involved in MiRNA biogenesis and processing in human cells

(circulating miRNAs), particularly in blood, plasma, serum, and saliva. Therefore,they have potential to serve as biomarkers for specific physiological and pathological conditions such as cancer, transplant rejection, cardiac injury, infection, and others.[21] These circulating miRNAs are highly reliable and protected from endogenous RNAse activity, being bound to lipoproteins such as High-density lipoproteins (HDL), associated with Argonaute 2 (Ago2) protein, or packaged into microparticles (such as exosome-like particles, microvescicles, and apoptotic bodies).[22]

MicroRNAs as biomarkers for periodontitis

Evidences linking periodontal disease and systemic health problems such as diabetes, cardiovascular disease, rheumatoid arthritis and adverse pregnancy outcome, are mounting in the recent years, indicating the importance of early detection and treatment of periodontal disease. The development of diagnostic tools to detect the presence and activity of periodontal disease is therefore of high importance.

Periodontal disease severity has traditionally been assessed using clinical parameters like pocket probing depth, clinical attachment loss, bleeding on probing, and radiographic determination of alveolar bone loss. Most of these techniques lack the capacity to identify highly susceptible patients at risk for disease progression.[23]

MicroRNAs have been studied in association with periodontal diseases. Invitro, in vivo animal and in vivo human studies have detected miRNAs that could possibly serve as diagnostic markers for periodontal diseases. The in vivo human miRNA studies related to periodontal disease are summarized in Table 1. Most of these studies have been carried out in gingival tissue samples. Five most commonly detected and validated miRNAs associated with periodontal disease in these studies were miR-142-3p, miR-146a, miR-155, miR-203, and miR-223. These miRNAs may play important role and thus could become potential markers for periodontal disease.

Salivary miRNA biomarkers are emerging as tools for the detection of oral cancer and systemic diseases. Various studies have been carried out to illustrate the potential of saliva as a non-invasive diagnostic tool for the detection of oral cancerous and pre-cancerous conditions,32-34 but no published literature could be found for periodontal disease. Salivary microRNA detection in periodontal disease is a promising concept which will have huge potential in the field of periodontal diagnostics.

Conclusion

Newer high through put techniques such as Next generation sequencing have enabled researchers to characterise miRNA patterns in body fluids such as

Table 1: In vivo human miRNA studies related to periodontal disease

Author and year	Sample source	Method of miRNA detection	Results
Lee et al.(2011)[24]	Gingival tissues	miRNA PCR array	6 miRNAs upregulated
Xie et al. (2011)[25]	Gingival tissues	miRNA Microarray	91 miRNAs up-regulated, 34 miRNAs
down-regulated			
Stoecklin-Wasmer et al.(2012) [26]	Gingival tissues	miRNA Microarray	4 miRNAs up-regulated, 7 miRNAs down-regulated
Perriet al. (2012) [27]	Gingival tissues	miRNA PCR array	11 miRNAs up-regulated
Ogata et al. (2014)[28]	Gingival tissues	miRNA Microarray	17 miRNAs upregulated and 22 miRNAs downregulated
Kalea et al. (2015) [29]	Gingival tissues	GeneChip miRNA Microarray	13 miRNAs upregulated and 22 miRNAs downregulated
Motedayyen et al. (2015) [30]	Gingival tissues	Real time PCR assay	Upregulated miR-146a
Saito et al.(2017) [31]	GCF	miRNA PCR system	40 miRNAs upregulated and 46 miRNAs downregulated

serum, plasma, and saliva on a large scale. Fundamental research into the mechanisms underlying control and activity of miRNAs will facilitate the identification of links between various diseases. In addition, more efficient computational prediction models and improved bioinformatic pipelines will allow optimal use of miRNA datasets. The combination of new diagnostic tests with saliva sampling will provide easy, rapid, testing, and will enable large scale and follow-up studies to be conducted at lower costs than when using traditional blood or tissue samples. With the introduction of new and improved techniques, more individuals susceptible to periodontal disease and with poor prognosis for other conditions will be detected early, allowing patient-focused clinical treatments.

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JSPIK

Effect of irradiation of 810nm laser on bone for 60 sec: A rabbit histological study

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ABSTRACT

Introduction and Objective: In last decade, low level laser therapy has been evaluated for stimulation and acceleration of bone formation. In spite of promising results, biphasic 'dose' response remains. Moreover, the use of single session of low level laser on healing of bone is not explored thoroughly. The aim of this study was to determine the optimal 'dosage' for formation of bone using diode laser of 810nm under single irradiation.

Materials and Methods: Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 8 months old for the study. Femur was chosen as site of surgery. The centre of the femur was drilled using implant osteotomy drills to the size of 2.8mm in width and 6mm in depth. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were, wavelength of 810nm, power of 90mW, time of 60 seconds in continuous mode using the disposable fibre of 300µmm diameter in punctual contact. Contra lateral femur was used as a control and the laser was sham treated. At the end of 2 weeks samples were collected from the surgical area and slides were prepared and analysed histologically.

Results: At 14th day, the lased group showed extensive haemorrhage, abundant amount of inflammatory cells. There was no evidence of bone formation in lased site.

Conclusion: The results of the present study using 810nm, 90mW, for 60 sec for single session induced extensive hemorrhage in two weeks compared to non-laser irradiated group. So the above parameters can be regarded as inhibitory dose for formation of bone.

Introduction:

Bone loss is a major concern in dental specialty and it could happen due to trauma, surgery or from any pathological disease. Sometime bone defects may be too large to large to repair it or there are conditions where you require regeneration of bone like in osteotomy sites or in periodontal defects. Bone repair is possible by several methods, which include bone grafts, guided tissue regeneration, bone morphogenic proteins etc. Recently methodology in use is LLLT.

Healing of bone is slow and has to go through several phases and it depends on nature of trauma. Low-level laser therapy (LLLT) applications on bone are still controversial. Several studies have shown positive effect on healing of bone in conditions like extraction sockets, orthodontic treatment and dental implant placement. It is more effective in initial stages of healing compared to later stages as it possibly increase the adenosine triphosphate and

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alkaline phosphatase levels in the tissues.[1-4]

The nature of LLLT depends on irradiation time, mode, total dose, energy density and intensity. This further requires an investigation to determine an optimum dosage for regeneration of bone. So the aim of the present study is to assess the effect of 810nm diode laser on regenerative capacity of bone for 60 sec histologically.

Materials and Method:

The study was carried out adhering to guidelines of the CPCSEA and institutional ethical committee. Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 10 months old for the study. Before the surgery the animals were anesthetized using ketamine (15mg/Kg) and xylazine (10mg/Kg.). Antibiotic prophylaxis started prior to the surgery (ceftriaxone 500mg). Femur was chosen as site of surgery, the skin overlying the femur was shaved and disinfected with Povidone-Iodine solution. 3 cm incision was given upto the bone on the lateral aspect of the femur exposing the underlying fascia and bone. The muscles were retracted using surgical elevators. The centre of the femur was drilled using implant osteotomy drills and widened 0.8 mm under copious irrigation of normal saline. Final dimension of osteotomy site was 2.8mm in width and 6mm in depth. (Fig.1) The sites were cleaned with irrigation of saline. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were wavelength of 810nm, 90mW, for 60 seconds in continuous mode using the disposable fibre of 300µmm diameter with noninitiated tip. Multiple points were chosen for irradiation of laser along apex, mid-medial, and mid-lateral of osteotomy site. (Fig.2,3) Contralateral femur was used as a control and the laser was sham treated. After the irradiation, the surgical site was sutured in layers using catgut 2.0 (Fig.4). Post operative antibiotics (ceftriaxone 500mg) was continued twice daily IM for 5 days. They were kept in the animal house and were fed vegetable diet. At the end of 2 weeks all animal were euthanized using high dose of Thiopental sodium. Samples were collected from the surgical area and restored in 10% buffered formalin. After that specimens were subjected to 4 % EDTA for demineralization.

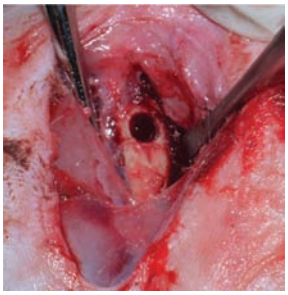

Fig 1: Femur

Fig 2: Diode laser

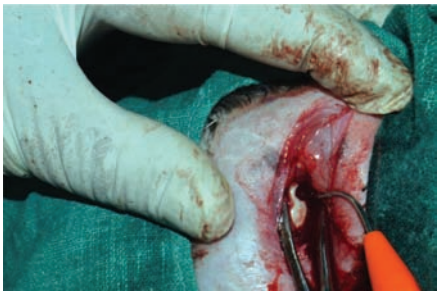

Fig 3: Application of Laser

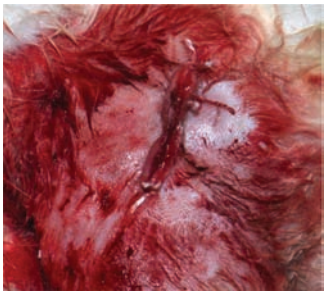

Fig 4: Suturing done

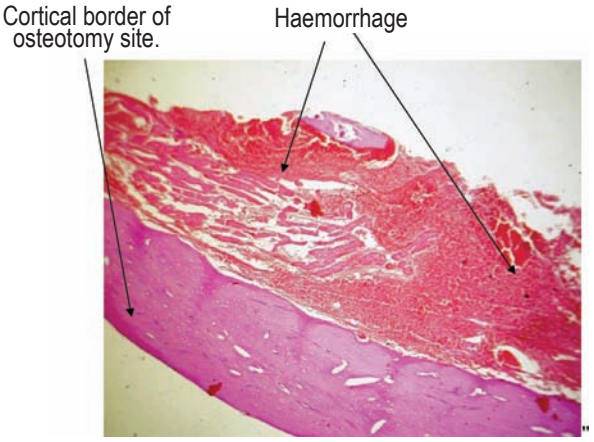

Fig 5: Laser Irradiated

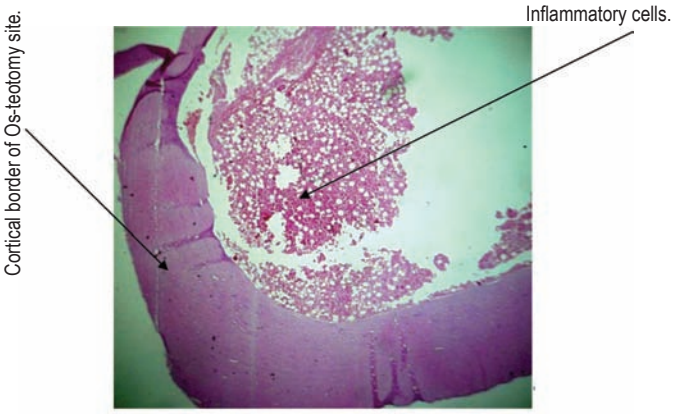

Fig 6: Control

Longitudinal cuts were made to divide the bone in to two halves. Then slides were prepared and stained with Haematoxylin and eosin (H.E. stain, Merck). All tissue specimens were examined by light microscopy and were assessed histologically using compound microscope (Olympus). (Fig 5,6)

Results:

After 2 weeks, the lased group section shows, areas of bone and connective tissue. Bone is compact in nature with osteocytes within lacunae and haversian system, bone resorption is seen in some areas. Connective tissue shows muscle fibers, hemorrhage and inflammatory cells and areas of haemorrhage, abundant amount of inflammatory cells. There was no evidence of bone formation in lased site. Non-lasedsection shows; compact bone with the bone marrow. Bone is compact in nature, with osteocytes enclosed in lacunae and haversian systems, bone marrow is filled with adipose tissue, and scanty lymphomatous tissue. There was no evidence of bone formation in nonlased site.

Discussion:

Photobiomodulation or low-level laser therapy (LLLT) is a drug-free, noninvasive, and safe clinical application of light, usually produced by low- to mid-power lasers or light emitting diodes (LED), with a power output in the range of 1–500 mW to a patient to promote tissue regeneration and healing, reduce inflammation, and relieve pain. The light is in the visible (red) or near infrared (NIR) spectrum (600–1000 nm) and achieves an average power density between 1 and 5 W/cm2[5].

A biphasic response has been demonstrated many times in LLLT research and the "Arndt-Schulz Law" is frequently quoted as a suitable model to describe dose dependent effects of LLLT.[6,7,8,9,10,11,12] Arndt-Schulz law states that weak stimuli slightly accelerate vital activity, stronger stimuli raise it further, but a peak is reached and even stronger stimuli suppress it, until a negative response is finally achieved. In the context of LLLT the increasing "stimulus" may be irradiation time or increased irradiance. A "biphasic" curve suggests that if insufficient energy is applied there will be no response (because the minimum threshold has not been met), if more energy is applied then a threshold is crossed and biostimulation is achieved but when too much energy is applied then the stimulation disappears and is replaced by bioinhibition instead.[13]

The aim of our study was to investigate the effect of GaAlAs low level diode laser under single irradiation on healing of bone. Dereci Ö et al.[14] histologically compared effects of blue light-emitting diode (LED) light (400-490nm) and Ga-Al-As low-level diode laser light (980nm) on bone regeneration of calvarial critical-sized defects in rats. Thirty Wistar Albino rats were included in the study. The experimental groups were as follows: blue LED (400-490nm) group; 980-nm low-level laser light group (LLL); and no-treatment, control group (CL). A critical-sized defect of 8mm was formed on calvaria of rats. Each animal was sacrificed 21 days after defect formation. Calvarias of all rats were dissected and fixated for histological examination. Histomorphometric measurements of total horizontal length of the newly produced bone tissue, total vertical length of the newly produced bone tissue, and diameter of the newly produced longest bone trabecula were performed with a computer program in micrometers. There was a statistically significant increase in the total horizontal length and total vertical length in LL and LED groups compared to that in the CL group ($P<0.05$), while there was no statistical difference between LED and LL groups ($P>0.05$). A statistically significant difference was observed in the longest bone trabecula and LL groups compared to that in CL ($P<0.05$), but not between LED-CL and LED-LL groups ($P>0.05$). Authors concluded that blue LED light significantly enhances bone regeneration in critical-sized defects when compared with CL group, but does not have a statistically significant effect on bone regeneration when compared with 980-nm low-level laser light. Angelo LuizFreddo et.al[15] conducted a study on influence of Magnetic field and Laser therapy on the quality of mandibular bone during distraction osteogenesis in rabbits. The sample consisted of 18 rabbits divided into 3 groups of 6 animals each: control, MF exposure (briefly, magnetized gold-coated washers were placed next to the distractor device), and LLLT exposure (830 nm applied every 48 hours over 4 points [dose, 5 J/cm2] during the consolidation period). The same distraction osteogenesis protocol was used in all 3 groups (0.5 mm every 12 hours for 1 week). Quantitative microscopic analysis of sections stained with hematoxylin and eosin showed

a statistically significant difference in the amount of newly formed bone in the MF group compared with the LLLT group (P = .006). The number of cells with more than 3 argyrophilicnucleolar organizer regions also was significantly different between the LLLT and control groups (P=.038). Distraction osteogenesis effectively promoted bone lengthening. The LLLT group exhibited a larger amount of newly formed bone and a larger number of osteoblasts in the cell division phase, but the difference was not statistically relevant compared with the control group. Bosco AF et.al[16] analyzed histomorphometrically the effect of low-level laser therapy (LLLT) on bone formation process in surgically created critical-size defects (CSDs) treated with bovine bone graft (BBG) and its influence over particles' resorption of BBG. A 10-mm diameter CSD was surgically created in the calvaria of 64 male rats, which were distributed into 4 experimental groups: the C group (control), only blood clot; the LLLT group, LLLT (GaAlAs, 660nm) and blood clot; the BBG group, CSD filled with BBG; the BBG/LLLT group, LLLT and CSD filled with BBG. Animals were euthanized at either 30 or 60 days post-operation. A histological analysis was performed. Additionally, the percentage of newly formed bone area (NFBA) and remaining particles areas (RPA) of BBG were histometrically evaluated and data statistically analyzed. The LLLT (5.82±2.05; 7.34±1.01) group presented significantly greater NFBA when compared to the C group (1.61±0.30; 5.59±0.94) at 30 and 60 days post-operation ($p<0.05$). The BBG/LLLT group (7.39±1.45; 9.44±2.36) presented significantly greater NFBA than the BBG group (3.85±1.56; 8.02±0.63) at 30 and 60 days post-operation ($p<0.05$). There was no significant difference in the mean percentage of implanted material RPA between the BBG and the BBG/LLLT groups. Authors conclude that LLLT can improve bone formation process in CSD filled or not with BBG in ratcalvaria, but it is not able to accelerate particles resorption of this material in the interior of bone defect. Hamad SA[17] evaluated the effect of low-level laser therapy on healing of extracted tooth socket of healthy rabbits. The sample of this study was 20 male rabbits of 2-2.5 kg weight with age range of 8-12 months. Right and left lower first premolar teeth were extracted. The extraction sockets of lower right first premolar were irradiated with 0.9W gallium-aluminum-arsenide (GaAlAs) diode laser for 5 min, immediately after extraction and then every 72 h for the next 12 days. The extraction socket of left side were not exposed to laser and served as a control. The animals were sacrificed after 7, 14, 30 and 45 days and the experimental and control sockets were removed from the harvested mandibles and prepared for haematoxylin and eosin staining and Masson's stain. The prepared slides were examined under light microscope for histological and histomorphometric examination. The histological examination showed that diode laser-treated sockets demonstrated early formed new bone with faster maturation of primary bone to secondary bone as compared to non-treated control sockets. Histomorphometric analysis revealed a statistically significant increase in the density and volume of trabecular bone in laser-treated sockets than control sockets. Authors concluded that diode laser application to tooth extraction socket has a positive effect on bone formation. Our study is in disagreement with above authors. Our study showed that irradiation with diode laser for 60 sec inhibited the resolution of inflammation and filled the up the marrow spaces with the inflammatory cells. There was no bone formation in healing bone irradiated with laser. Our study results are in agreement with Bouvet-Gerbettazet al.[18] and Coombe et al.[19] showed no beneficial effect of laser on the proliferation and differentiation of osteoblasts. The variation in the outcomes of LLLT application on bone repair may be attributed to different physical parameters used by different researchers. The main benefits of therapeutic laser seem to derive from the administering of doses, since there have been unfavorable results when the laser is used at higher levels or for extended periods of time[20]. Barbosa et al.[21], in evaluating the effect of different wavelength and time of application of LLLT in an experimental fracture model in rats confirmed that the positive effect of LLLT in bone repair is time- and wavelength-dependent. Wavelengths of diode lasers used in studies with positive results were 635, 685, 810, and 830 nm. Satisfactory results are obtained when the number of applications ranges from two to twelve low-energy sessions[22,23]. Jakseet al.[24] did not confirm the positive LLLT effect on bone healing in cancellous sinus graft, because of it inadequate irradiation power (4 J/cm2) and absorption of most irradiated light by thick sinus cortical bone and deep

Figure 1

sinus of the sheep. It may be assumed that low doses of laser induce bone formation, so high doses should produce increased bone volume. But this assumption most of the times is not correct. By contrast LLLT in high doses will lead to intracellular high numbers of reactive oxygen species and apoptosis of cells.[25]

The different doses, application protocols and experimental models complicate comparisons between the studies. Many variables may affect the LLLT biostimulatory effects such as, laser wavelength, energy, exposition time, power, and the biologic state of the cell, power density, beam profile, energy density, number and frequency of treatment and duration of treatment.[26]

Conclusion:

The results of the present study using 810nm, 90mW, for 60 sec for single session inhibited the formation of collagen and bone formation in two weeks. So the above parameters can be regarded as inhibitory dose for formation of bone.

Further scope of the study is to evaluate the effect of low level laser therapy using different energy densities for short duration of time in continuous wave.

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JSPIK

Gingival biotype-a review

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ABSTRACT

The term gingival biotype refers to the soft tissue quality of profile pertaining to that tooth. Various tissue biotypes respond differently to inflammation and to surgical and restorative treatment; consequently, it is important to identify tissue biotype before treatment. These factors dictate the disease progression, treatment outcome and prognosis. Periodontal biotype evaluation is an important parameter in establishing patient expectations in many complex esthetic procedures by allowing the clinician to predict therapeutic outcome. This paper reviews the various clinical implications and methods of assessment of gingival biotype.

Keywords: Biotype, Scalloped, Transgingival methods, Ultrasonography, Calipers

Introduction

The term gingival biotype pertains to the quality of the soft tissue profile surrounding the teeth. The gingival morphology plays an important role in determining the final esthetic outcome. Therefore during treatment planning, it is important to recognize differences in gingival tissue. In 1969, Ochsenbein & Ross indicated that there were 2 main types of gingival anatomy— flat and highly scalloped.[1] The authors reported that flat gingiva was associated with a square tooth form, while scalloped gingiva was associated with a tapered tooth form and proposed that the gingival contour closely mimics the contour of the underlying alveolar bone. The termperiodontal biotype was used later by Seibert & Lindhe, who classified the gingiva as either thin-scalloped or thick-flat.[2]

The teeth associated with flat gingiva are of square shaped with pronounced cervical convexity. The gingiva of such individuals is wide with more volume, the contact areas between the teeth are large and more apically located, and the interdental papillae are short. Teeth associated with scalloped gingiva have slender teeth, tapered crown form, delicate cervical convexity and minute interdental contact areas that are located close to the incisal edge.

The following characteristics have been assigned to each biotype. (Oschenbein and Ross, 1969)

Thin and scalloped	Thick and flat
Delicate soft tissue	Thick heavy soft tissue
Highly scalloped gingival tissue	Flat gingival contour
Usually slight gingival recession	Gingival margins usually coronal to CEJ
Dehiscence and fenestrations are more	Thick, flat osseous contour
Minimum zones of keratinized gingiva	Wide zone of keratinized gingiva
Small incisal contact areas	Broad apical contact areas
Triangular tooth form	Square tooth form
More prone to recession	Insult results in pocket depth or rebundant tissue
Subtle cervical concavity with proximal contact close to incisal edge	Bulbous convexities in cervical third of facial surface

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Factors affecting gingival biotype and gingival bioform

The different parameters which affect the two morphologic types (biotype and bioform) are gingival complex, tooth morphology, contact points, hard and soft tissue considerations, gingival bioform, and biotype. Hence, a clinician's knowledge of anatomy, form, and function of the dentition is of paramount importance in achieving optimal treatment outcomes. It has long been known that clinical appearance of healthy marginal periodontium differs from subject to subject and even among different tooth types.[3] It has been suggested that many features are directly genetically determined, whereas other morphologic characteristics of the periodontium seem to be influenced by tooth size, shape and position, and biological phenomena such as growth or ageing.[4] Gingival thickness affects the biotype of the gingiva, whereas, crown width (CW): Crown length (CL), papilla height, and gingival width are responsible for determining the gingival bioform.

Crown width –crown length ratio

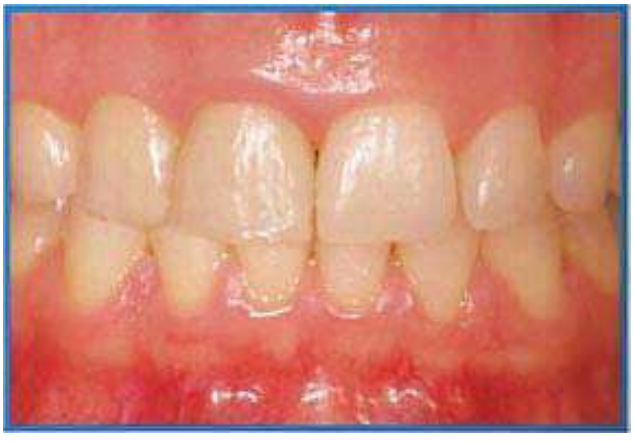
There was a tendency for a flat gingival architecture to have a lower tooth height-to-width ratio, while a scalloped gingival architecture was associated with a higher tooth height-to-width ratio[5]. It has been observed that individuals having a tapered tooth form usually have a thin, scalloped gingival architecture, and clinically; this has been associated with an increased susceptibility to recession. This theory was further supported by studies demonstrating that central incisors with a narrow crown form had a greater prevalence of recession than incisors with a wide, square form.[5] However, Eger et al on the other hand, failed to observe a meaningful influence of CW/CL ratio on gingival thickness.[6]

Gingival thickness

It has been suggested that different gingival entities have different tooth shapes. Many studies have examined the correlation between the tooth shape and gingival biotype. Sanavi (et.al,) claimed that the thick and flat periodontal tissues have a rectangular tooth shape, and the thin scallop shaped periodontal tissues have a triangular tooth shape.[7] Olsson and Lindhe reported that long and narrow crowns have thin periodontal tissues and a high likelihood of having gingival recession compared to the thick gingival biotype, suggesting a relationship between the tooth shape and gingival biotype.[8]

Age and sex

The thicker biotype is more prevalent in male population while the female population consists of thin, scalloped gingival biotype. On comparing, the prevalence of gingival biotypes between different age groups, the thick flat biotype is seen in younger individuals while older age group shows thin scalloped gingival biotype. Vandana (et.al) in their study on gingival thickness showed thicker gingiva in younger age group and stated that decrease in keratinization and changes in oral epithelium may be the contributing factors.[9] Chang in his study stated that an inverse relationship has been found to be existing between papilla height and age.[10] Sanavi (et. al) in their review article described that the inter-root bone is more in the thinner biotype.[7] This, in turn, can cause more recession. They also stated that the interproximal papilla does not cover the spaces between two teeth in thinner biotype as compared to thick biotype. This could possibly relate to increased amount of recession and also the presence of thin biotype in older age group. Chow (et.al) also evaluated various factors associated with the appearance of gingival papillae and found significant associations



with age and the crown form and gingival thickness.[11] Olsson (et. al) documented that the central incisors with narrow tooth form had greater amount of recession when compared to incisors with square form.[8] With age, the interdental papilla recedes; this explains the greater frequency of thin biotype seen with older age group. Anterior teeth with narrow zones of attached gingiva are frequently encountered in children. Maynard and Ochsenbein suggested that newly erupted permanent teeth with narrow attached gingiva may run a greater risk of gingival recession. In the permanent dentition, the gingival problems are often noticed in the age when children are candidates for orthodontic treatment, and considerable attention has been focused on various therapeutic measures.[12]

Papilla height

The interdental papilla occupies the interdental or embrasure space and acts as a barrier to protect underlying periodontal structures and also plays an estheticrole. The distance from the contact point to the interproximal alveolar crest has been identified as a critical factor in the presence of a complete papilla, with nearly 100% of papillae filling the gingival embrasure completely if contact point-bone crest distance is ≤5 mm.[13] Factors contributing to the presence and absence of dental papillae are crestal bone height and/or interproximal distance. Many other factors that might influence papillary appearance such as tooth form/shape, gingival thickness and keratinized gingiva/attached gingiva width, distance from the contact point to the bone crest, inter-radicular distance, size of the embrasure space, have never been fully examined, but these have been listed to be relevant factors. There is a positive correlation between gingival thickness and papilla fill. Decrease in papilla height is observed with thin biotype. Limited blood supply is believed to be one of the major reasons why papilla preservation and regeneration are difficult. Thicker tissue may resist collapse and contraction due to increased vascularity and extracellular matrix volume. In addition, thicker keratinized gingival epithelium may be more resistant to physical damage and bacterial ingress. Therefore, thick gingival biotype has been considered more favorable for achieving optimal aesthetics.[14]

Gingival width

The attached portion of the gingiva is clinically defined as the distance from depression below the projection on the external surface of the gingival sulcus to the mucogingival junction. There is no minimum width of keratinized or attached gingival tissue necessary to maintain health, provided plaque control is adequate; however, sites with narrow keratinized gingiva have been associated with increased recession when exposed to mechanical trauma or poor oral hygiene, and also, it has been suggested that a wide zone of keratinized and attached gingiva is more desirable than a narrow zone or a total lack of such a zone, because a wide zone would better withstand gingival inflammation, trauma from mastication, tooth brushing and forces from muscle pull and orthodontic procedures.[15]

Influence of gingival biotype on periodontal treatment planning and outcomes

The gingival morphology plays an important role in determining the final esthetic outcome. Therefore during treatment planning, it is important to recognize differences in gingival tissue. Different gingival biotypes respond differently to inflammation, restorative, trauma and parafunctional habits. A gingival thickness of >2 mm was considered as thick tissue biotype and a gingival thickness of <1.5 mm was referred as thin tissue biotype. The initial gingival thickness is significant as it may predict the outcome of root coverage procedures and restorative treatments.[16]

Treatment Outcome:

The gingival thickness affects the treatment outcome possibly because of the difference in the amount of blood supply to the underlying bone and susceptibility to resorption. Periodontal surgical techniques can significantly improve the tissue quality and treatment outcome. Periodontal surgical techniques can enhance tissue quality resulting in a more favourable treatment outcome. Soft tissue grafting in areas of thin biotypes can enhance the quality of the gingival tissue. The best way to convert a thin soft tissue to a thick biotype is through sub epithelial connective tissue grafting. Various other soft tissue augmentation procedures include modified roll technique and use of acellular dermal matrix. Tissue keratinization can be improved by oral physiotherapy. Understanding periodontal biotype is also of importance in orthodontic treatment. Alteration of mucogingival dimensions may occur during orthodontic treatment resulting from proper tooth position within the alveolar bone. It has been demonstrated that the gingival tissue with a little

horizontal diameter in the presence of a dental plaque, is more susceptible to apical migration of connective tissue attachment with marginal gingivae specially near teeth under the influence of orthodontic force. However, in cases with thin gingiva caused by the prominent position of the teeth, there is no need for pre orthodontic gingival augmentation procedures. The recession and bone dehiscence will decrease when the tooth is moved in a more proper position within the alveolar bone.[17]

Alveolar Bone:

Kan (et. al) in 2003 measured the dimensions of the gingiva by bone sounding at the mesiobuccal and distobuccal aspects of maxillary anterior teeth. Bone sounding determines the distance between the soft tissue margin and the crest of the bone and, hence, provides an estimate that is about 1 mm greater than that obtained in a regular probing pocket depth measurement. The authors reported that the thickness of the gingiva varied between subjects of different gingival biotypes. Thus, the height of the gingiva at the buccal approximal surfaces in subjects who belonged to the flat biotype was, on average, 4.5 mm, while in subjects belonging to the pronounced scalloped biotype the corresponding dimension on an average of 3.8 mm was significantly smaller. This indicates that subjects who belong to the flat biotype have more voluminous soft approximal tissues than subjects who belong to the pronounced scalloped biotype.[18]

Pontoriero (et.al) in 2001 performed evaluations of the reformation of the gingival unit at the buccal aspect of teeth exposed to crown lengthening procedures using a denudation technique.[19] At the 1 year follow up examination after surgery the regain of soft tissue measured from the level of the denuded osseous crest was greater in patients with a thick biotype than in those with a thin biotype. No assessment was made of the bone level change that had occurred between the baseline and the follow up examination. It must, however, be anticipated that some bone resorption had taken place during healing and that the biologic width of the new connective tissue attachment had been re established coronal to the level of the resected osseous crest.

Root Coverage:

Thickness of tissues in the recipient site and the donor site are key factors in treating mucogingival defects. In cases involving root coverage procedures, a flap thickness of 0.8-1.2 mm produced more predictable outcomes. An initial gingival thickness was found to be the most predictable factor for predicting the success of complete root coverage procedures. There is a correlation between flap thickness and complete root coverage. A thick tissue has an increased blood supply that will enhance the revascularization of grafts, leading to increased healing and graft incorporation and hence there are more chances of complete root coverage in thick biotype.[20]

Crown Lengthening Procedures:

Thick gingival tissues are more resistant to mucosal recession or mechanical irritation and are capable of creating a barricade to conceal restorative margins. With crown lengthening procedures, it is often difficult to predict the final position of the soft and hard tissues, due to the fact that each time when a flap is reflected, there is at least 0.5–0.8 mm of bone loss. There could be undue gingival recession following surgery. So before placement of permanent restoration in the anterior region a healing period of at least six months is desirable. In an extremely thin gingival tissue, soft tissue grafting is recommended 6–8 weeks prior to surgical crown lengthening to improve the thickness of the keratanised tissue.[21]

Ridge Preservation:

Thick biotypes show greater dimensional stability during remodeling compared to thin biotypes. A thin gingival biotype is associated with a thin alveolar plate. More ridge remodeling has been found in thin biotype when compared with thick periodontal biotype. Ridge preservation should be considered for most thin biotype cases. Preservation of alveolar dimensions such asatraumatic extraction, socket preservation or ridge preservation techniques after tooth extraction are critical for achieving optimal esthetic results in thin biotypes[22].

Implant Therapy:

Evidence suggests that the percentage of the success rate of immediate implants in anteriors is more in individuals with thick biotypes. However in patients with thin biotypes the frequency of gingival recession is high following implant restoration. The thicknesses of the crestal bone on the buccal aspect significantly influence remodelling during the initial

four month healing period after immediate implant placement. A delayed implant must be considered when there is not enough soft and hard tissue thickness. However immediate implants can be considered with predictable results in thick biotypes.[23]

Maxillary Sinus lining:

Aimetti (et.al) in 2008 took maxillary mucosal biopsies from the sinus floor during otorhinolaringologic surgical interventions, and measured gingival thickness in the area of the maxillary anterior teeth.[23] The authors reported that the average thickness of the Schneiderian membrane was 0.97 ± 0.36 mm. Patients with thick gingiva had a sinus mucosa that was 1.26 ± 0.14 mm thick, compared to 0.61 ± 0.15 mm thickness among patients with thin gingiva. The results showed that gingival thickness is a reliable factor for predicting sinus membrane thickness. However research on this is still in its infancy.

How to measure

Many Invasive and non invasive methods have been used to evaluate the thickness of facial gingival and other parts of the masticatory mucosa[24]. The method of assessment of gingival biotype ranges from assessment with periodontal probe, probe transparency visual examination, ultrasonic devices or radiographic methods to conventional histology on cadaver jaws, injection needles, transgingival probing, histologic sections, cephalometric radiographs and cone beam computed tomography.[25]

Visual Evaluation

Simple visual evaluation is used in clinical practice to identify the gingival biotype; however, it may not be considered a reliable method, as it cannot be used to assess the degree of gingival thickness.[26]

Probe Transparency

The gingival tissue's ability to cover any underlying material's coloris necessary for achieving esthetic results, especially in cases of implant and restorative dentistry, where subgingival metal restorations are used widely. Using a metal periodontal probe in the sulcus to evaluate gingival tissue thickness is the simplest way to determine gingival biotype; with a thin biotype, the tip of the probe is visible through the gingiva. This method is minimally invasive and can be performed routinely during periodontal probing procedures.[27]

Modified Caliper

A tension free caliper can only be used at the time of surgery and cannot be used for pre-treatment evaluation. A 2010 study by Kan (et.al)of the facial gingival biotype in maxillary anterior teeth compared visual evaluations, the use of a periodontal probe, and direct measurements with a tension free caliper. The authors reported a statistically significant difference between visual assessment and both the periodontal probe and the tension free caliper; however, there was no statistically significant difference when comparing the periodontal probe assessment and the tension free caliper. Based on these results, a periodontal probe in the sulcus is an adequately reliable and objective way to evaluate tissue thickness, whereas visual evaluation of the gingival biotype by itself is not as reliable as the periodontal probe or the tension-free caliper.[28]

Transgingival Probing

Gingival thickness can be measured by using a periodontal probe; however, such measurements can be affected by the precision of the probe, the angulation of the probe, and the distortion of the tissue during probing.[29]

Ultrasonic Devices

A 1971 study by Kydd (et.al) was the first to measure the thickness of palatal mucosa using an ultrasonic device. Ultrasonic devices appear to be the least invasive method and offer excellent validity and reliability. However, such devices are no longer available commercially; in addition, they make it difficult to both determine the correct position for accurate measurement and successfully reproduce measurements.[30]

Cone Beam Computed Tomography (CBCT)

CBCT scans have been used extensively for hard tissue imaging because of their superior diagnostic ability. CBCT measurements may be a more objective method than direct measurement. Thickness of alveolar bone plate surrounding the tooth is associated with the type of biotype. Thick buccal bony plate usually corresponds to thick gingival biotype. Measuring the thickness of bony plate by CBCT can be a noninvasive method for assessing type of gingival biotype.[31]

Puffed cheek method

Dvorak (et.al) in 2013 described this method in a case series where they assessed the thickness of

mucosa by using computed tomography with splint placed to localize the exact position by a marker points. Marker points were placed at four sites. The Four sites were evaluated two central incisors and two first molars. Patients were asked to puff out cheeks because, Computed tomography scans with distended cheeks provide a more detailed evaluation of mucosal surfaces of the oral cavity than conventional CT scans.[32]

Conclusion

Different tissue biotypes exhibit different pathological responses when subjected to inflammatory, traumatic or surgical insults as they have different gingival and osseous structures. These different responses, dictate different treatment modalities. The current periodontal surgical techniques have the potential to improve the tissue quality, thereby enhancing the restorative environment. With the knowledge of the nature of tissue biotypes, clinicians can employ appropriate periodontal management to minimize tissue resorption and provide more favourable results after treatment. So by taking into consideration the gingival tissue biotypes during treatment planning, more appropriate strategies for periodontal management may be developed, resulting in more predictable treatment outcomes.

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Does a relationship exist between anaemia of chronic disease and chronic periodontitis?- A review

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ABSTRACT

The current paradigm for the pathogenesis of chronic periodontitis emphasizes on the immune-inflammatory nature of the disease. Inflammatory mediators produced in periodontal inflammation bring about a systemic challenge. Association between various systemic diseases and chronic periodontitis has been described in literature with varying evidences. The cytokines released as in response to periodontal inflammation induce changes in the iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin and the life span of red blood cells; all of which contribute to the pathogenesis of anaemia in chronic periodontitis. Observational and experimental studies are reported regarding the association between chronic periodontitis and anaemia of chronic disease. Aim of this review is to give an insight to the pathogenic mechanisms of anaemia of chronic disease and plausibility of relationship between anaemia of chronic disease and chronic periodontitis by an appraisal of the current literature.

Key words: Periodontitis, Anaemia of Chronic Disease, Erythropoietin, Hepcidin.

Introduction

Anaemia refers to a state in which the level of haemoglobin in the blood is below the reference range appropriate for age and sex. Factors like pregnancy and altitude also affects haemoglobin levels[1]. About 30% of the world population is anaemic[2]. In India, anemia is a common and serious health disorder among both sexes and all age groups, although it has a greater prevalence among women[3]. As per national family health survey (NFHS-4, 2015-16) in India, 53% of women and 23% of men aged 15-49 years are anaemic[3]. Based on aetiology, anaemia can be broadly classified into anaemia due to blood loss, increased rate of destruction of red blood cells and impaired red cell production. Anaemia of chronic disease comes under the category anaemia due to impaired red cell production[4]. Anaemia of chronic disease is the second most prevalent anaemia after anaemia caused by iron defiency[5].

Anaemia of chronic disease (ACD)

ACD is defined as the anaemia that occurs in the setting of chronic infection, inflammatory conditions or neoplasia that is not due to marrow deficiency or other disease and occurs despite of the adequate iron stores and vitamin[6]. It is also called as anaemia of inflammation[7]. Underlying causes of ACD are given in Table I.

Aetiopathogenesis

ACD is immune driven, cytokines and cells of the reticuloendothelial system induce changes in the iron homeostasis, the proliferation of erythroid

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progenitor cells, the production of erythropoietin and the life span of red blood cells;all of which contribute to the pathogenesis of anaemia[8]. Various pathogenic mechanisms are illustrated in Figure I.

a) Dysregulation of iron homeostasis

The hallmark of ACD is the development of the disturbances of iron homeostasis,with increased uptake and retention of iron within cells of the reticuloendothelial system (RES). This produces diversion of iron from the circulation into storage sites of the RES. Subsequently iron availability for erythroid progenitor cell is decreased and development of iron-restricted erythropoiesis[8]. The acquisition of iron by macrophages takes place mostly through erythrophagocytosis[9] and the transmembrane import of ferrous iron (Fe2+) by the protein divalent metal transporter 1 (DMT1)[10]. Pro-inflammatory cytokines like TNF –α and interferon - γ and bacterial lipopolysaccharide up-regulate the expression of DMT1[11]. These stimuli also induce retention of iron in macrophages by down-regulating the expression of ferroportin, thus blocking the release of iron from macrophages[11]. Ferroportin is a transmembrane exporter of iron, responsible for the transfer of absorbed Fe2+ from duodenal enterocytes into circulation (Figure I, panel A and C).

The identification of Hepcidin, an acute phase protein synthesised by liver enlightened on the relationship of immune response to iron homeostasis and anaemia of chronic disease. Hepcidin expression is induced by pro-inflammatory cytokines, especially interleukin-6 (IL-6) and lipopolysaccharides[12]. Hepcidin binds to ferroportin on small intestinal enterocytes and macrophages and internalise the ferroportin and inhibiting the export of iron from these cells into blood. Consequently iron remains trapped inside the cells in the form of ferritin and hypoferraemia and elevated ferritin levels results[13]. A recently identified gene, haemojuvalin may act in concert with hepcidin in inducing these chages[14]. The disruption in iron homeostasis and subsequent limitation of the availability of iron for erythroid progenitor cells impair the proliferation of these cells by negatively affecting haembiosynthesis (Figure I, panel B)

b. Impaired proliferation of erythroid progenitor cells

Pro-inflammatory mediators like Interferon-α, β and γ(IF-α, β and γ), TNF –α and IL-1 inhibits the proliferation and differentiation of erythroid precursors-erythroid burst forming units and erythroid colony forming units. IF-γ appears to be the most potent inhibitor. The underlying mechanism may involve cytokine mediated induction of apoptosis which may in part related to the production of ceramide, the down-regulation of erythropoietin receptor expression on progenitor cells, impaired production and activity of erythropoietin and a reduced expression of other pro-haematopoietic factors such as stem cell factors. Additionally, direct toxic effects of cytokines erythroid progenitor cells are also reported[15] (Figure I, panel E).

c. Blunted erythropoietin response

Erythropoietin, a hormone produced by kidney, regulates erythroid cell proliferation centrally. Erythropoietin response in ACD is inadequate for the degree of anaemia in most conditions. The cytokines IL-1 and TNF-α directly inhibit erythropoietin expression in part, due to cytokine mediated production of reactive oxygen species which in turn affects the binding affinities of erythropoietin reducing transcription factors and also damage erythropoietin-producing cells. The responsiveness of erythroid progenitor cells to erythropoietin appears to be irreversibly related to the severity of underlying chronic disease and the amount of circulating cytokines, because in the presence of high concentration of IF-γ and TNF–α, much higher concentration of erythropoietin are required to restore the formation of erythroid colony forming units[15] (Figure I, panel D).

Chronic periodontitis

Periodontitis is an immune-inflammatory disease characterised by host-mediated destruction of bone and connective tissue that support the teeth[16]. Chronic periodontitis is the most common type of periodontitis[17]. Periodontal disease results from a complex interplay between subgingival microbiota and host immune-inflammatory events that develop

in the gingival and periodontal tissues in response to the challenge produced by bacteria. There was a significant conceptual shift in the aetiopathogenesis of periodontitis. Earlier it was thought that periodontitis is an infection caused by subgingival bacteria. Later it was proposed that bacteria induce tissue destruction indirectly by activating host defence cells, which in turn produce and release mediators that stimulate the effectors of connective tissue breakdown[18]. The prevailing paradigm was that bacteria were necessary, but not sufficient, to cause periodontitis. The bacteria and their products evoke an immune-inflammatory reaction in the host tissue which leads to the production of cytokines, most characteristically tumour necrosis factor- alpha, IL-1 and IL-6. Although this process is intended to eliminate the microbial challenge, it often results in damage to the host tissue[19].

Bacteria and their products stimulate accumulation of polymorphonuclear leukocytes at the gingival site and subsequently the release of proteolytic enzymes and reactive oxygen species (ROS). ROS include molecules like hydrogen peroxide, hypochlorous acid, singlet oxygen, and ozone, and their excessive production by polymorphonuclear leukocytes is one of the pathologic features in the periodontal lesion.[20] ROS can cause periodontal tissue destruction by oxidizing DNA, proteins, lipids, and important enzymes such as antiproteases, stimulating proinflammatory cytokines release through depletion of intracellular thiol compounds, and activating nuclear factor-kappa B (NF-ϰB)[21]. Inflammation is a key feature of the pathogenesis of ACD and periodontitis. The inflammatory response in periodontitis is characterized by dys-regulated secretion of host-derived mediators of inflammation leading to tissue break down. Interleukin- 1β (IL-1β), Interleukin-6 (IL-6), prostaglandin E2 (PG-E2), Tumour Necrosis Factor-α (TNF- α), Receptor Activator of NF-ϰB Ligand (RANKL) and matrix metalloproteinases (MMP-s) as well as T cell regulatory cytokines are the most extensively studied cytokines in periodontitis.

The sulcular epithelium acts as a protective barrier and prevents entry of microorganisms and other irritants into the systemic circulation. The host-microbial interaction in periodontitis leads to ulceration of sulcular epithelium. The ulcerated

Table I Underlying causes of ACD8

Associated disease	Estimated prevalence (%)
Infections (acute and chronic) (viral infections, including HIV infection, bacterial, parasitic, fungal)	18 – 95
Cancer (haematological, solid tumour)	30 – 77
Auto-immune (rheumatoid arthritis, systemic lupus erythematosus and connective-tissue diseases, vasculitis, sarcoidosis, inflammatory bowel disease)	8 – 71
Chronic rejection after solid-organ transplantation	8 – 70
CKD and inflammation	25 – 30

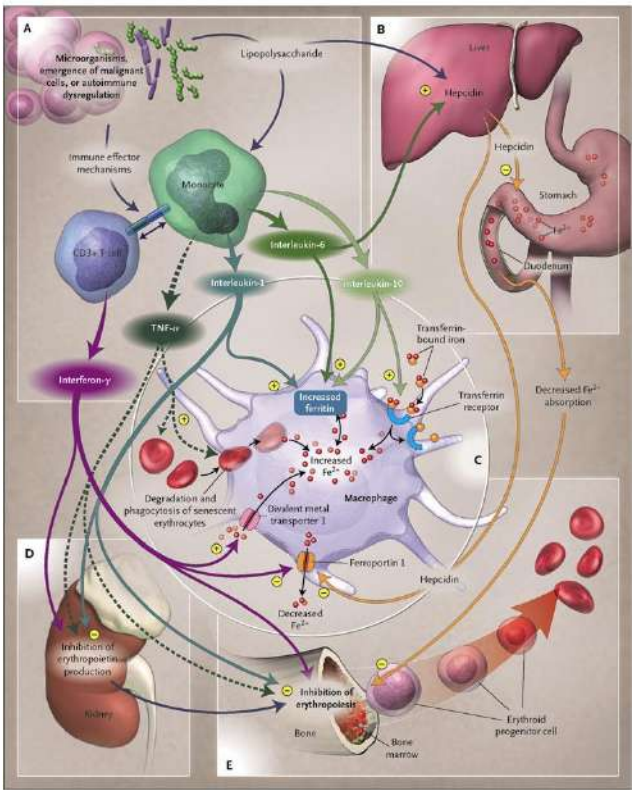

Figure I: Pathogenesis of anaemia of chronic disease[8] (figure taken from N Engl J Med 2005;352:1011-23)

epithelium acts as a portal of entry for the bacteria and cytokines to enter into the systemic circulation. Bacteremia has been observed in patients with periodontitis and has been directly related to the severity of inflammation[22]. Serum levels of CRP,an acute phase protein synthesised by liver, is also elevated in periodontitis; which also points to the systemic burden of inflammation due to periodontal disease[23]. Epidemiological studies suggest that periodontitis is associated with an increased risk for systemic diseases like diabetes mellitus, prediabetes[24], rheumatoid arthritis[25], cardiovascular diseases, cerebrovascular ischemia and atherosclerosis[26]. These associations indicate that periodontitis has systemic effects and that most likely signs of systemic inflammation must be present.

ACD and chronic periodontitis share common mechanism of pathogenesis via inflammatory cytokines and ROS. Hence, a relationship is plausible between ACD and chronic periodontitis.

Pro-inflammatory cytokines like TNF–α and interferon γ and bacterial lipopolysaccharide in periodontitis may upregulate the expression of DMT1, protein involved in the import of ferrous ion (Fe2+) into macrophages and subsequent retention of iron in these cells which may lead to anaemia. Moreover, pro-inflammatory cytokines, especially interleukin-6 (IL-6) and lipopolysaccharides induces the expression of hepcidin, the key regulatory protein which accounts for the characteristics of ACD. TNF-α and IL-6 are the main inducers of acute phase proteins, including hepcidin and C-reactive protein (CRP). Serum levels of CRP is also elevated in periodontitis[23].

Pro-inflammatory mediators released as a result of inflammation in periodontitis, like Interferon-α, β and γ(IF-α, β and γ), TNF –α and IL-1 inhibits the proliferation and differentiation of erythroid precursors which in turn leads to anaemia. The cytokines IL-1 And TNF-α directly inhibit erythropoietin expression and subsequent reduction in the reticulocyte count and development of anaemia[5].

ROS released by host PMNs as part of the inflammatory response in periodontitis may also down-regulate the erythropoietin expression resulting in anaemia. These mechanisms suggest that like any other chronic inflammatory disease,chronic periodontitis may also be a causative agent in the development of anaemia of chronic disease[15].

A few early reports in the literature have observed anemia in periodontitis[27,28,29] but except for the report by Siegel in 1945[29], the authors have been lead to believe that perhaps anemia was one of the causes of destructive periodontitis, rather than to regard this phenomenon as a consequence. Mild to moderate anemia has been reported in subjects with rheumatoid arthritis[30,31]. The cause for ACD is most likely multi-factorial, however it is currently thought

Table II Investigations to differentiate anaemia of chronic disease from iron deficiency anaemia[13,42,8]

Blood parameters	Iron deficiency anaemia	Anaemia of chronic disease	Both conditions
Hb	↓	↓	↓
Iron	↓	↓	↓
Ferritin	↓	↑/normal	↓/Normal
TIBC	↑	↓	↓
Transferrin saturation	↓	↓	↓
Soluble transferrin receptor	↑	↓/normal	↑/normal
sTFR/logferritin ratio	↑	↓	↑
Hepcidin	↓	↑	Normal
GDF15	Normal	↑	↑
Inflammatory markers	Normal	↑	↑

that pro-inflammatory cytokines from a given chronic disease process, such as rheumatoid arthritis, may down-regulate the erythropoiesis in bone marrow[32].

VidyaNaik et al in 2010[33] observed that the mean values of hemoglobin, red blood cells, packed cell volume, and mean corpuscular hemoglobin concentration were significantly lower, while the mean corpuscular volume of erythrocytes and erythrocyte sedimentation rate were significantly higher in chronic periodontitis subjects compared to those in controls, indicating mild anemia in chronic periodontitis subjects.

A. R. Pradeep et al in 2010[34] conducted a study in chronic periodontitis subjects with anaemia and concluded that non-surgical periodontal therapy can improve the anaemic status of patients with chronic periodontitis with greater improvement in females.

Ranjan Malhotra et al in 2012[35] conducted an interventional study in patients with anaemia of chronic disease and chronic periodontitis and concluded that non-surgical periodontal therapy improve the anaemic status in chronic periodontitis subjects.

HN Santosh et al in 2015[36] conducted a study in chronic periodontitis subjects and concluded that chronic generalized periodontitis, by means of an inflammatory process, influences various hematologic parameters and may result in anaemia.

There have been conflicting findings as far as the Hb levels are concerned in periodontitis patients. Wakai K et al in 1999[37] observed no significant correlation between hemoglobin level and CPITN scores. Anne Havemose-Poulsen et al in 2006[38] in an observational study found that there is no statistically significant difference in erythrocyte count between chronic periodontitis subjects and healthy controls. Rajashri A et al in 2014[39] observed that there was no significant difference in hemoglobin levels in patients with chronic periodontitis compared to healthy controls, though periodontitis group showed lower erythrocyte count and mean corpuscular hemoglobin concentration (MCHC), and increased total leukocyte count (TLC) and neutrophil, lymphocyte, and eosinophil count, compared to the healthy control group. S. Latha, et al in 2014[40] evaluated the blood parameters in chronic periodontitis subjects and healthy controls and concluded that both the groups showed iron deficiency and macrocytic anemia, No statistically significant correlation was found to exist between periodontitis and erythrocyte count, levels of hemoglobin, hematocrit, and serum ferritin.

Differentiating between Iron deficiency anaemia and ACD

It is often difficult to distinguish ACD associated with a low MCV from IDA. Examination of the marrow may ultimately be required to assess iron stores directly[13]. Laboratory investigations to differentiate ACD from IDA are given in Table II.

Patients with IDA and concurrent inflammation present a diagnostic dilemma. IDA and ACD are differentiated primarily by estimates of iron status such as ferritin, serum iron, total iron-binding capacity (TIBC) or transferrin, and transferrin saturation. However, these tests are directly affected by chronic disease, hindering the clinical interpretation of results[41]. These changes make the interpretation difficult in iron-deficient patients who have an accompanying infection or inflammatory disease. In contrast, soluble transferrin receptor (sTfR) has been shown to be an indicator of iron deficiency and is unaffected by concomitant chronic disease and inflammation. Calculating the sTfR/log ferritin index (sTfR Index) provides an estimate of body iron over a wide range of normal and depleted iron stores. The sTfRIndex thus takes advantage of the reciprocal relationship between two variables influenced by iron deficiency, an increase in sTfR and a decrease in the ferritin concentration. Use of sTfR and the sTfR Index improves detection of IDA, particularly in situations where routine markers provide equivocal results[41].

Management

The anaemia observed in ACD is frequently mild, and correction may not always be necessary. Several reasons for attempting to correct the anaemia present. Firstly, anaemia may be deleterious in itself, with effects on the cardiovascular system needed to maintain tissue oxygen supply. Secondly, anaemia may be associated with a poorer prognosis in many

chronic disease states although whether anaemia plays a causative role in determining prognosis is open to debate. Thirdly, treatment may improve the quality of life for patients living with chronic conditions. As ACD occurs as a manifestation of underlying disorder, resolution of the disorder generally help to improve the ACD. ACD in chronic periodontitis is found to be improved by proper periodontal therapy.

Conclusion

Anaemia of chronic disease is common form of anaemia occurring in chronic inflammation,infe ction,malignancies or other chronic conditions. It is a cytokine-mediated anaemia; acute phase protein hepcidine plays a pivotal role in its etiopathogenesis. As periodontitis is a chronic inflammatory disease, anaemia is also reported in chronic periodontitis. Anaemia is usually mild but the major huddle lies in the differentiation of this type of anaemia from iron deficiency anaemia. Management of periodontitis usually leads to improvement in haemoglobin levels. Hence the dental and medical fraternity should spread more awareness about the relationship between these two chronic diseases and should join hands to manage these patients in an interdisciplinary manner.

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A novel approach in successful management of labial cervical vertical groove – a case report

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ABSTRACT

Radicular grooves are developmental anomalies of maxillary incisors, which contribute to localized periodontitis resulting in loss of anterior teeth. Labial-cervical-vertical groove (LCVG), which starts on the cervical enamel and extends to the radicular surface, has also been described as a notch. Clinician's awareness of existence of such grooves may help to avoid misdiagnosis and improper treatment of these patients. Knowledge of these factors is very critical for early detection and treatment to prevent further attachment loss. This case report describes the successful management of Labial Cervical Vertical Groove using Biodentin, Platelet Rich Fibrin + Bone Graft along with Amniotic membrane.

Keywords: Labial cervical vertical groove (LCVG), Platelet rich fibrin (PRF), Biodentin, Amniotic membrane

Introduction

Periodontal disease is a host modulated multifactorial infectious disease resulting in inflammation within supporting structures of the teeth, which leads to progressive attachment loss, bone loss and hence the loss of tooth. Although biofilm[1] is the most common etiological factor, various anatomical, morphological[2] and other iatrogenic factors can also result in initiation of periodontal destruction. These factors can cause alteration in dentogingival relationship thus making more prone for the virulent periodontal pathogens to harbor which results in the development of site specific localized Periodontitis.

Diverse morphological tooth anomalies are found in various textures, shapes and prevalence. Toothe -born deformities[3] which includes tuberculum carabelli, dens invaginatus, taurodontism, shovel - shaped incisors, variations in number and shape of cusps, palatogingival groove have been exclusively investigated.

Labial cervical vertical groove (LCVG), runs vertically from the crown surface to the root, starting at the enamel on the crown cervix and extends apically, crossing the cementoenamel junction.[4] It resembles a short furrow and has also been described as "notch". The presence of labial cervical groove on the enamel surface of the maxillary central incisors was determined by Brin I and Ben Bassat Y in 1989.[5] The etiology of this defect is unknown but the defect apparently arises from the infolding of the enamel epithelium and Hertwig's root sheath during odontogenesis,[6] and some have speculated that this is an aborted attempt towards formation of an additional

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root.[7] Some other authors said that this represents the mildest form of dens invaginatus,[8] or Enamel hypoplasia, caused by impaired function of ameloblasts during tooth development.[9] This may also due to alteration in genetic mechanisms and racial link or nutritional issues; trauma or injury.[10]

Severity of LCVG was ranked in three stages[11]: [1] a mild subgingival shallow groove below the marginal gingiva that can be felt only by probing; [2] a moderate groove that can be detected with the eyes, extending subgingivally and additionally supragingivally on the labial crown surface, not more than 2 mm from the marginal gingiva in the incisal direction; [3] a severe defect extending supragingivally more than 2 mm the marginal gingiva on the labial crown surface and further subgingivally. When an LCVG was present, the gingival contour was described in three categories: normal coverage - the gingiva covers the groove with no change in the regular shape of the gingival margin; partial coverage - the gingiva partially covers the groove with mild change in the contour; irregular coverage - the gingiva covers the groove with a severe change in the contour.

Knowledge of these factors is very critical for early detection and treatment to prevent further attachment loss. These grooves are often overlooked as etiologic factor hence this case report describes the successful management of Labial Cervical Vertical Groove using Biodentin, PRF + Bone Graft along with Amniotic membrane.

Case report

A 29 year old male patient reported to the department of Periodontology, with a chief complaint of pus discharge from maxillary right central incisor with dull intermittent pain. There was no relevant medical history. Patient was non smoker and had not

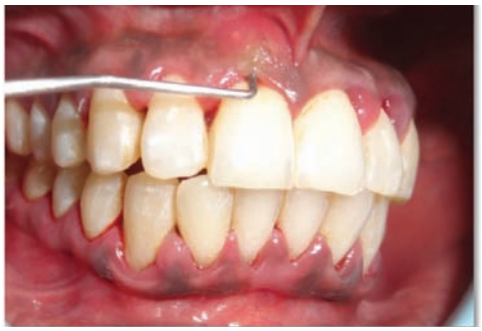

Fig - 1. Pre - op photo showing 10mm probing depth in midfacial aspect of groove in relation to 11.

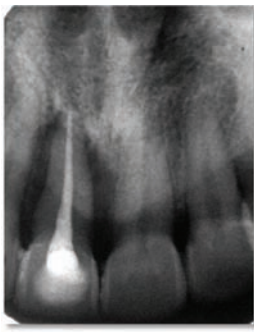

Fig - 2. Pre - op IOPA with RCT Done

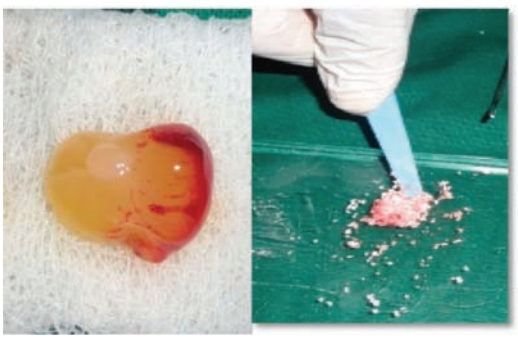

Fig - 3 - PRF and PRF + Bone Graft

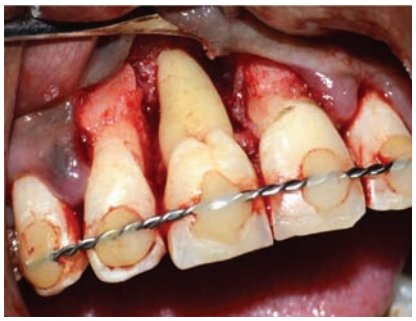

Fig. 4 - Labial groove extends onto the root surface after flap elevation.

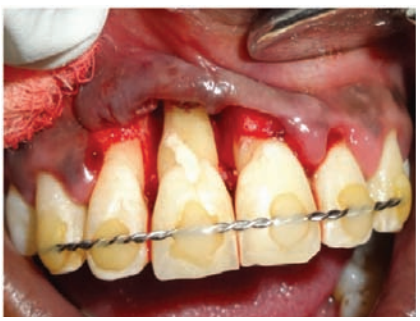

Fig - 5 Biodentin placed in a groove

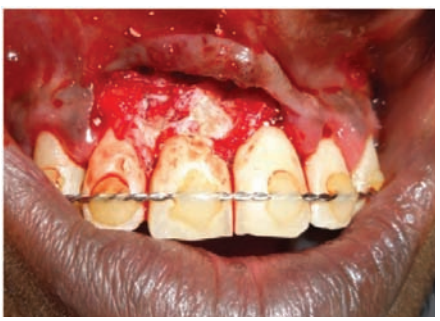

Fig - 6 Bone Graft with PRF + Amniotic membrane placed

taken any long term medications. On careful clinical examination a groove was found in the mid-labial aspect of 11. On periodontal examination a localized gingival inflammation was seen with the accumulation of plaque and calculus with respect to 11, 12, 21 and 22. A probing pocket depth of about 10 mm was seen in relation to 11 (Fig. 1). Fremitus was found to be negative which showed that there was no trauma from occlusion. On thermal testing the tooth was found to exhibit delayed response. Patient exhibited tenderness on percussion in relation to 11. A periapical radiograph was taken in which a radiolucency extending from cervical region of 11 extending till the apical third suggesting of localized bone loss. Patient advised for routine Blood Investigations. Patient platelet count, haemoglobin, random blood sugar, bleeding time, and clotting time were assessed and found to be within normal limits. Endodontic therapy - Root canal treatment was done in relation to 11 (Fig. 2). The surgical periodontal therapy was planned as pocket persists after 4 weeks.

The PRF was prepared in accordance with the protocol developed by Choukroun et al. Prior to surgery, intravenous blood from anti cubital vein was collected in the 10 ml of sterile tube without an anticoagulant and centrifuged immediately. Blood was centrifuged using a table top centrifuging machine for 12 min at 2,000 rpm.

PRF was easily separated from RBCs using a sterile tweezer just after removal of PPP and then transferred on to the gauze. The PRF was then mixed with bone graft (Fig. 3). The Amniotic membrane

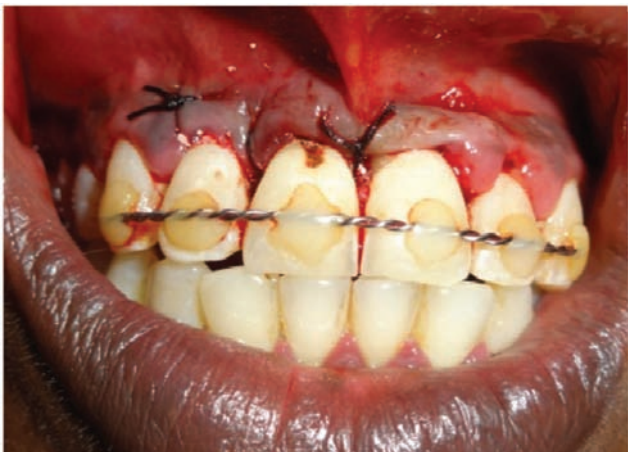

Fig - 7 Sutures Placed

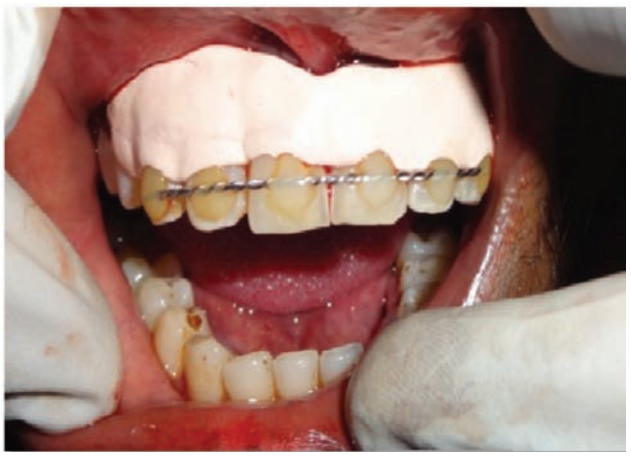

Fig - 8. Coe – pak placed

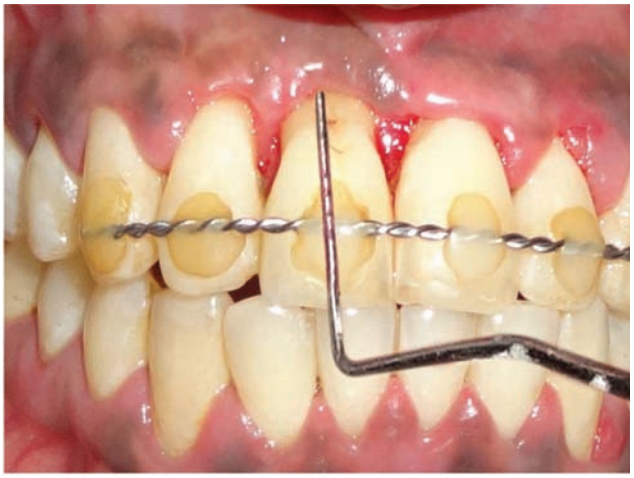

Fig 9 - Post op photo showing 2mm probing depth in midfacial aspect of groove in relation to 11.

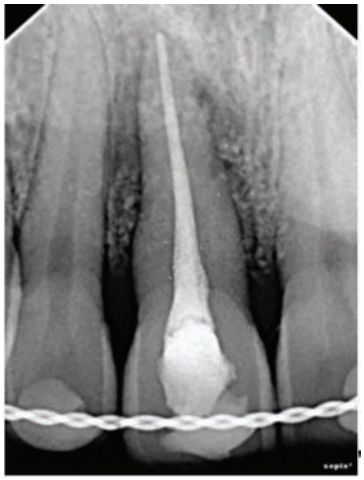

Fig - 10 - (3 - Months) Post Op Iopa

was purchased from Tissue Bank, Tata Memorial Hospital, Mumbai.

Surgical procedure

The Root canal treatment was done in relation to 11 and Splinting was done before the surgery. Intra-oral antisepsis were performed using 0.2% chlorhexidine digluconate rinse and iodine solution was used to carry out extra oral antisepsis for the patient. Following administration of local anesthesia, sulcular incisions given in relation to 12, 11 and 21 and a vertical incision given in distal to 12 made and muco-periosteal - full thickness flap [kirkland] was reflected. Meticulous defect debridement and root planning was carried out with the help of area specific Gracey curettes (Fig. 4). Saucerization done using diamond coated ultrasonic tip and biodentin is placed on the groove (Fig. 5). The direct examination after debridement, confirmed the presence of bony defect. PRF + bone graft was filled into the infrabony defect and amniotic membrane is placed to stabilize the graft (Fig. 6). The mucoperiosteal flap were repositioned and secured in place using 3-0 non absorbable black silk surgical suture. The independent sling sutures were placed (Fig. 7). After that immediate post operative IOPAs were taken. Periodontal dressing was placed (Fig. 8). The suitable antibiotics and analgesics (amoxicillin 500 mg thrice for 5 days and Aceclofenac 100 mg twice for 3 days) were prescribed along with 0.2% chlorhexidine mouth wash twice daily, for one week. Periodontal pack and sutures were removed after one week and saline irrigation was done.

Results

The patient was monitored at regular intervals and was under maintenance therapy. At the end of 3 months and 6 months, clinical examination and intra-oral periapical radiographs of the treated area were taken. The clinical measurements were repeated and compared to the baseline values. On examination during subsequent follow-ups, treated area showed satisfactory healing without any post operative complications. LCVG was completely sealed off. There is a reduction in pocket depth of 10 mm to 3mm (Fig - 9) and bone defect depth was initially about 11 mm and it is reduced to 4 mm and bone fill was evident for about 7 mm. Periodontal health was stable and bone regeneration was noticed in radiograph (Fig. 10). Patient was satisfied with the result. At 3 months, biodentin placed on the coronal aspect of groove is replaced with tooth coloured Glass Ionomer Cement.

Discussion

Periodontitis is a polymicrobial infectious disease resulting in loss of connective tissue attachment to root surfaces. The developmental groove (Radicular groove) is one of the reason for bone loss and localized periodontitis[12]. There are different treatment modalities have been proposed[9]: Subgingival curettage; Odontoplasty; Saucerization and filling of grooves with restorative materials; Combined endodontic and periodontal treatment.

In this present case, the defect was of a severe type and it was successfully managed with combined Endodontic and Periodontal treatment using Biodentin, PRF + Bone Graft along with Amniotic membrane.

"Biodentine - A Bioactive Cement" has been used for the sealing of the groove due to its superior handling characteristics and excellent biocompatibility and act as an ideal biomaterial for periodontal repair and/or regeneration in defects that has been created by pathways of communication between the periodontium and the pulp.

Biodentine is preferred over MTA due to its better handling characteristics and short setting time because when using MTA in sealing of palatoradicular groove it is difficult to control moisture during its setting which leads to degradation and poor marginal seal In the case reports done by Johns et al[13] and Naik et al[14], on palatoradicular groove, biodentine has been successfully used in sealing the groove as it has a reduced setting time, better mechanical properties and with no need for surface conditioning and bonding that makes it easy to handle. Thus in the present study Biodentine has been opted to seal the groove.

PRF was first described by Choukroun et al. It is considered as a second-generation platelet concentrate and has been used in various surgical procedures in an attempt to enhance wound healing[15]. Compared to other platelet concentrates, PRF releases the growth factors at a sustained rate over a longer period, thereby optimizing wound healing.[16] Recently, PRF has also been shown to stimulate the growth of osteoblasts and periodontal ligament cells,

both of which are significant for the regeneration of periodontal defects[17]. In the cases done by Shah et al[18] and Nadig et al[19] reported the effectiveness of PRF for the treatment of intrabony defect associated with labial cervical vertical groove. Thus in the present study PRF was used successfully to treat intrabony defect associated with the groove.

Bone grafts are widely used in periodontal therapy and have been demonstrated to be safe and capable of inducing osteoconduction and osteoinduction. The graft material induces host undifferentiated mesenchymal cells to differentiate into osteoblasts with subsequent formation of new bone.[15] The case reports done by Kim et al[20] and Gupta et al[21] showed predictable clinical attachments when bone grafts used in intrabony defects associated with palatoradicular groove.

Simonpieri et al[22] has mentioned the advantages of using PRF along with bone graft. Fibrin clot serve as biological connector between bone particles and facilitates cellular migration, vascularization, and survival of the graft. The growth factors (PDGF, TGF-β, IGF-1) were gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing. The presence of leukocytes and cytokines in the fibrin network plays an important role in the self-regulation of inflammatory and infectious phenomena within the grafted material. Bansal et al.[23] and Khattar Sakshi et al.[24] had done a study to evaluate the efficacy of autologous PRF with the bone grafts, in the treatment of periodontal intrabony defects. They concluded that there is a significant improvement in the clinical probing depth, relative attachment level, and radiographical bone fill when PRF used with bonegraft. Thus in the present study we planned to use PRF with bonegraft which gave more predictable results.

The clinical application of amniotic membrane by fulfilling the current mechanical concept of GTR amends it with the modern concept of biological GTR. In this study Amniotic membrane is used to stabilize the graft. Amnion has shown an ability to form a nearly physiologic "seal" with the host tissue precluding bacterial contamination. Advantage of using Amniotic membrane over other membranes includes the following: (1)It not only maintains the structural and anatomical configuration but also contributes to the enhancement of healing through reduction of post operative scarring and subsequent loss of function and providing a rich source of stem cells[25]. (2) Amnion tissue contains growth factors that aids in the formation of granulation tissue by stimulating fibroblast growth and neovascularisation[26]. (3) It has an ability to decrease the host immunologic response via mechanisms such as localized suppression of polymorphonuclear cell migration.(4) Laminin 5 being the most prevalent plays a role in the cellular adhesion of gingival cells and concentrations of this glycoprotein is useful for periodontal grafting procedures[27]. Kumar et al[28] in his Randomized control clinical trial has showed that AM has the potential to function as a barrier for GTR and the unique properties associated with this material can augment its potential as a matrix for periodontal regeneration. Hence we used amnioitic membrane as GTR in our study.

In the present case, LCVG was sealed with Biodentin and associated intrabony defect was treated with Bone graft + PRF and Amniotic membrane was used to stabilize the graft. Periodontal condition was stable and bone regeneration was evident with significant attachment gain at grafted site.

Conclusion

This case reports the successful treatment of localized periodontal lesion on maxillary central incisor associated with Labial Cervical vertical Groove. These defects may provide seat for local factors to accumulate. Deep radicular grooves may predispose to pulp necrosis and establishment of combined endodontic periodontal lesions. Eliciting the clinical signs at the earliest, by careful examination, is of paramount importance in the treatment of radicular grooves.

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3D Scaffold in periodontal regeneration

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ABSTRACT

Tissue engineering was introduced in periodontal regeneration for the complete periodontal regeneration with new attachment. Scaffold is a "niche" for cells which provide a three-dimensional template structure to support and facilitate the processes of tissue regeneration. It facilitates the attachment, migration, proliferation, and three-dimensional (3D) spatial organization of the cell population. Three-dimensional scaffold which mimics the chemical composition, microstructure, mechanical and functional properties of native tissue is critical for regeneration of damaged tissue. The use of a prefabricated 3D scaffold, with the appropriate cells or instructive messages can overcome many of the limitations associated with current regenerative technologies. In this review article a comprehensive review of 3D scaffolds in periodontal regeneration is attempted.

Key words: Three-dimensional scaffold, tissue engineering, periodontal regeneration, compartmentalization, stem cell, three-dimensional printing

Introduction

The periodontium or surrounding tissues of the tooth comprises of gingiva, cementum, periodontal ligament (PDL), and alveolar bone. Periodontal disease is an inflammatory disease which is caused mainly by plaque biofilm. There is irreversible loss of tooth supporting apparatus in periodontitis which later leading to the loss of the tooth.

The treatment for periodontal disease is surgical and nonsurgical therapy where healing occur mostly by formation of long junctional epithelium which is susceptible to further breakdown. Complete periodontal regeneration with new attachment is the desired treatment outcome. Tissue engineering was introduced in periodontal regeneration to fulfill this desire. The triad of tissue engineering involves cells, signaling molecules, and scaffold/supporting matrices (Fig. 1). Cells act as the machinery for new tissue growth and differentiation. Growth factors and other biological molecules helps in maintaining the cellular activity and also provide stimuli for cells differentiation and support tissue neogenesis. Another critical factor in tissue regeneration is neovascularization.

Scaffolds provide a three-dimensional template structure to support and facilitate these processes that are critical for tissue regeneration.[4] Lots of research happening currently in all the three areas of tissue engineering.

Scaffold is a "niche" for cells. It facilitates the attachment, migration, proliferation, and three-dimensional (3D) spatial organization of the cell population. According to scientific literature, the term "scaffold" indicates a biomaterial that can give support. In this scenario, "support" is used to describe the biomaterial as a biological platform which facilitates the repair and restoration of the injured tissues during the healing process.[1,2] In tissue regeneration, ideal biocompatible scaffold should enhance cell adhesion and induce cell proliferation and differentiation. It should be osteogenic, osteoconductive and

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osteoinductive.[3] Three-dimensional scaffold which mimics the chemical composition, microstructure, mechanical and functional properties of native tissue is critical for regeneration of damaged tissue.[4] But currently scaffolds used in periodontal regeneration are two dimensional (2D) which has certain limitations. They are limited in reproducing the complex 3D environments existing in vivo. Cells grown on 2D scaffold are forced to adapt to an artificial flat, rigid surface and differ remarkably in their morphology, proliferation, and differentiation from those growing in 3D environments.

The use of a prefabricated 3D scaffold, with the appropriate cells or instructive messages (e.g. growth factors and matrix-attachment factors) incorporated into it, can overcome many of the limitations associated with current regenerative technologies. Scaffolds can be either acellular or cellular upon implantation. In the acellular type the recruitment of local stem cell or osteoprogenitor cells is promoted by overall architecture and geometry of the scaffold whereas in the cellular type, it involves implantation of a scaffold combined with stem cell and or/osteoprogenitor cells. 3D Printed biomaterials, can customize the desired size, architecture and configuration of a given defect.[6]

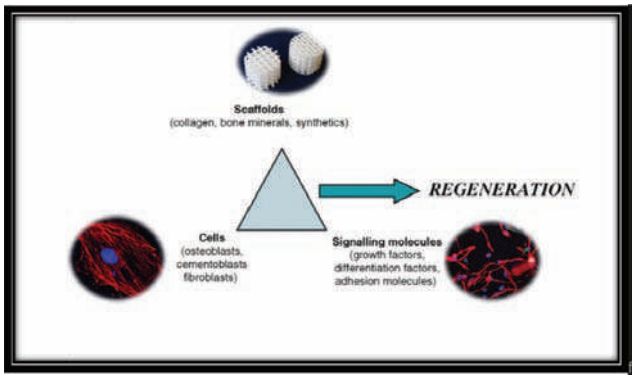

Figure 1. Triad of tissue engineering

There are various types of scaffold materials made available over years.[8] They are non-resorbable materials e.g.: -expanded polytetrafluoroethylene (ePTFE), ceramic and titanium mesh and resorbable materials e.g.: - alpha-hydroxyacids, polyglycolic acid, poly (l-lactic acid) and copolymers of poly (lactic-co-glycolic acid). Amino acid-based polymers e.g.: -collagen-like proteins and elastin-like proteins. Natural products e.g.:- collagen, hyaluronan, chitosan, gelatin and fibrin. Synthetic hydrogels e.g.: -poly (ethylene glycol) and poly (ethylene oxide). Matrix extracts e.g.: -Matrigel

Since the past 10 years several scaffold fabrication technologies have been using in periodontal tissue engineering including conventional prefabricated scaffolds, such as particulated, solid form, and injectable scaffolds that are adapted or administered into a periodontal defect. However, each of these methods has its own disadvantages; like the use of highly toxic solvents, retention of particles in the scaffolds matrix, thin structure limitation, irregularity in pores size and shape, smaller pore size and long processing time.[9] One of the important requirements of scaffold is compartmentalization of different tissues and subsequent re-organization and integration of fibrous and mineralized tissue, which is not facilitated by conventional scaffold. So through the provision of a prefabricated three-dimensional structure

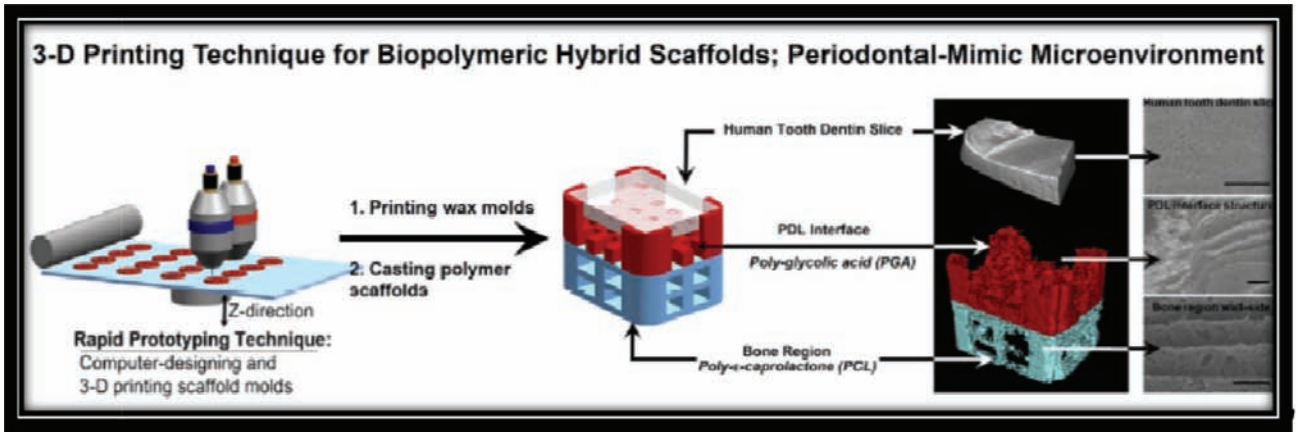

Figure 2. 3-D Printing using rapid prototyping technique. CAD-based 3-D wax molds manufactured and cast with biodegradable polymeric materials,including poly-glycolic acid (PGA) for the PDL interface in red and poly-"-caprolactone (PCL) for the bone region in blue.Courtesy of Park et al 2010.

we can overcome many of the limitations associated with conventional regenerative technologies. In this review article a comprehensive review of 3D scaffolds in periodontal regeneration is attempted.

3D scaffold: properties for periodontal regeneration

Ideal scaffolds must exhibit an adequate degree of hydrophilicity[15,16] roughness,[17] and specific surface topography.[18] The overall surface area, surface-to-volume ratio,and surface roughness is increased by nanotopography,[19] which enhance the adhesion between osteoblasts and the underlying scaffold surfaces.[20]

In case of alveolar bone regeneration applications an overall porosity of 40% can be applied.[21] Regarding pore diameter, a range between 150 μm and 500 μm facilitates vascularization and penetration of new tissues[22] without compromising the mechanical strength of the scaffold. Interconnection of the pore network is essential for cell growth into the interior of the scaffold to prevent core necrosis.[23]

It should demonstrate mechanical strength close to native tissues to support target cells, the surrounding tissues, and newly formed ones.[24,25] For this degradation rate of a scaffold should be in concordance with the remodeling processes of the target tissue[26] Degradation within 5-6 months is considered appropriate.[27]

Implanted scaffolds should be biocompatible and bioactive, and should not elicit cytotoxic reactions.[28] Another important property is "Compartmentalization" which is essential for controlling the spatiotemporal events which permits effective regeneration of the periodontal complex[29] and could prevent tooth ankylosis, achieved by compartmentalized formation of bone and functionally oriented periodontal ligament fibers (PDL) that are integrated over time.

Cell /tissue-scaffold interactions

One of the main goals of tissue engineering is optimizing cell-tissue-scaffold material interactions. Cell-to-cell contact between scaffold and cells is needed to stimulate the initial attachment. Cell spreading and cell growth is influenced by surface texture.[11] Surface roughness further influence the expression of adhesion proteins.[12] 3D printed scaffolds can be processed into a variety of shapes and sizes for ideal attachment and growth. In 3D scaffold surface area can be increased by high porosity and high interconnectivity which are essential for cell attachment and tissue in growth. Cell-material interaction is also influenced by the physical and chemical properties of scaffold surface.[13]

Recently, with the use of 3D bio-plotter system,

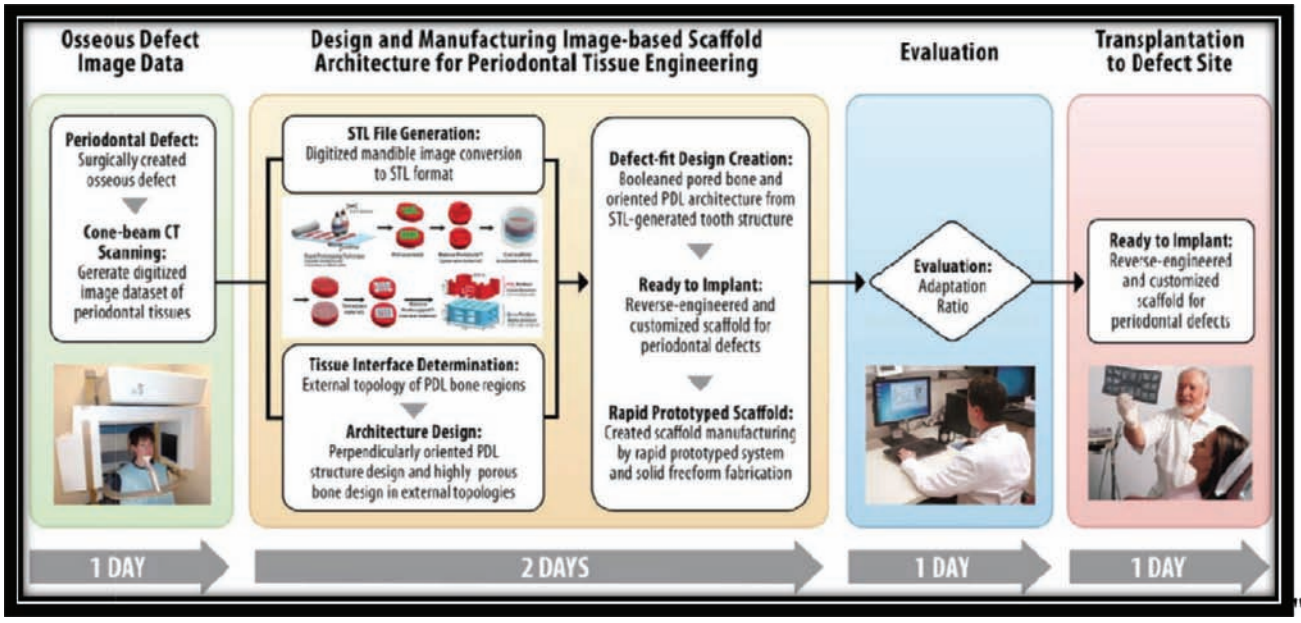

Fig 3. CADmodels are produced based on computed tomography (CT) scans of a patient-specific bone defect to develop a custom-madebone graft substitute. Courtesy of Park et al., 2014

different designs of the 3D scaffolds, different angles of rotation (45° and 85°), and scaffold characteristics promotes initial cell attachment and differentiation.[14]

Design Requirements

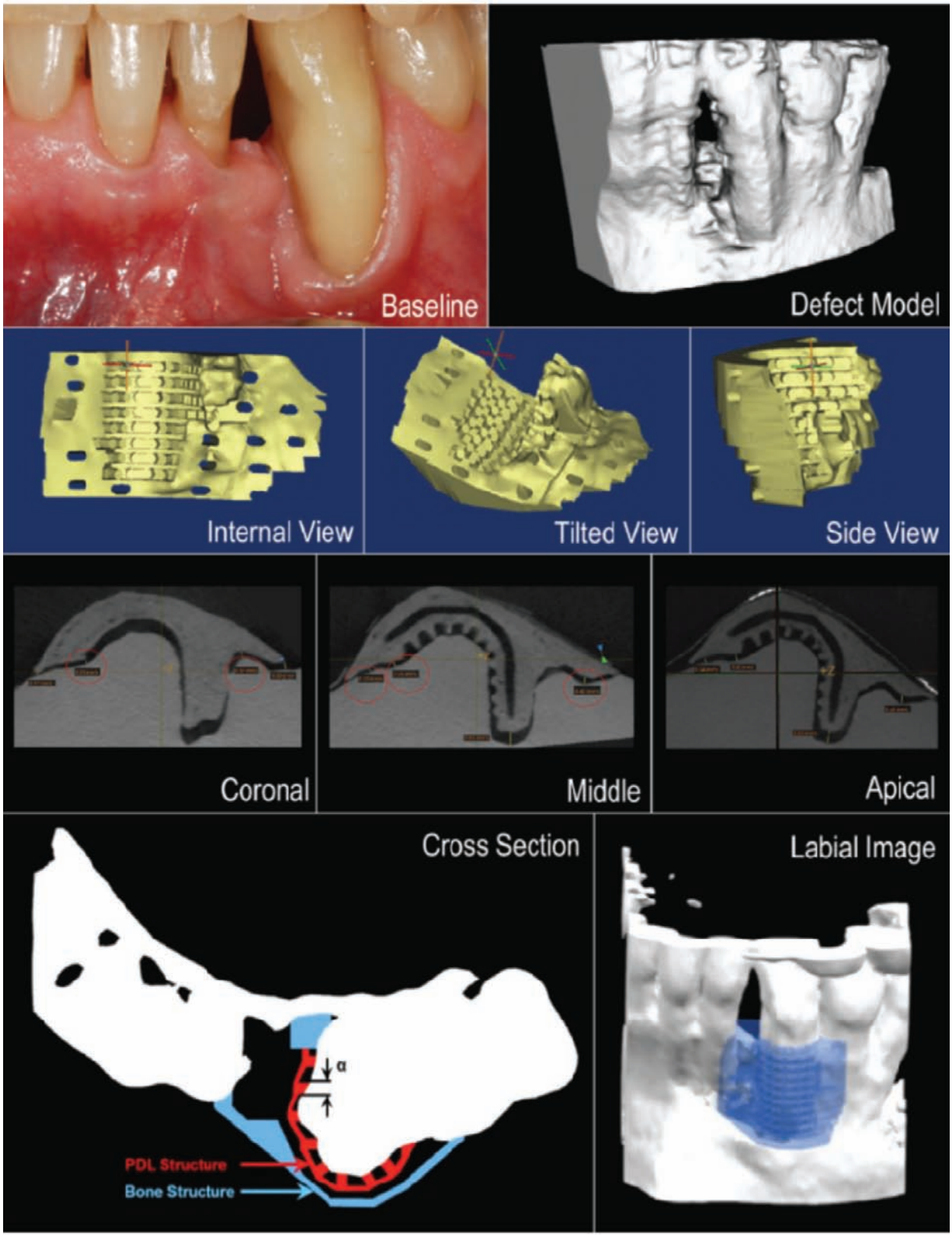

Figure 4.Customized scaffold was 3D printed using medical-grade polycaprolactone to fit the periosseous defect using a prototyped model of thedefect from the patient's cone beam computed tomography scan. Courtesy of Rasperini et al, 2015.

Space maintenance within the defect site

The material that is used as scaffold should have appropriate form and sufficient strength to allow placement into a defect and should prevents subsequent collapse of the overlying tissues into the defect site. So, it should follow principles similar to that of guided tissue regeneration and have similar design features.[29] So it is well understood that sufficient wound space and a suitable environment for regeneration will act synergistically to permit the uninhibited cascade of molecular and cellular events required for the regenerative process.

For adequate space maintenance the necessary design features will include ease of handling and shaping, sufficient rigidity to withstand soft tissue collapse into the defect, and an internal structure compatible with cell attachment and colonization, as well as permitting the in growth of tissues compatible with those to be regenerated.[30]

Barrier or exclusionary functions

The engineered tissues should act as a barrier by only permitting selective in growth of regenerative tissues and avoid in growth of unwanted tissues, for example, gingival epithelium and connective tissue.[30] Here comes important sealing role the epithelium into play. So, the principle aim of tissue engineering for the periodontium should not be total exclusion of the epithelium from the site but rather encouraging it to form a successful and rapid biological seal to protect the fragile underlying regenerating events.[31]

Biocompatibility and design features:

Tissue engineering scaffolds should be either biocompatible with the tissues which have to be regenerated or biodegradable, allowing for gradual replacement by regenerated tissue. The scaffold materials should always be free from transmittable disease and immunologically inert, and should not lead to exuberant inflammatory response. So the acceptance of the host to implanted material depends not only on the material used but also on the host reaction and the systemic health of the recipient.[32]

Incorporation of cells with an appropriate phenotype:

It should be possible to culture and subsequently incorporate cells with a periodontal-regenerative phenotype into a suitable biodegradable scaffold for immediate introduction into a periodontal defect. Now a days, viral vectors transduced into mesenchymal cells have been used to introduce specific molecules to wound sites with the intention of stimulating tissue regeneration. Cells from cementum,[33] periodontal ligament,[7] and bone[34] are potential sources for periodontal tissue engineering. The so-called progenitor cells that reside in these tissues needs to be evaluated whether it can be isolated and propagated in culture for future seeding.

Incorporation and bioavailability of instructive messages:

As the growth and differentiation factors are essential ingredients for tissue regeneration, the synthetic scaffold used for tissue engineering should be bioresorbable at the same time it should have suitable affinity for the adsorption of appropriate growth or differentiation factors as well as integrins, cell receptors and other instructive molecules normally found in regenerating tissues.[35] Notwithstanding this important requisite, choosing the correct agent or agents is a formidable task.

Biomaterials for 3D scaffolds fabrication

The biomaterials used in 3D scaffold fabrication are compatible with new scaffold fabrication techniques and can be mainly applied in regeneration.

Biodegradable Natural Polymers.

First biomaterials to be recruited in different clinical applications are natural polymers, which include proteins and polysaccharides because of their high biocompatibility, good cell recognition, enhanced cellular interactions in the surrounding environment,[37] and hydrophilicity.[38]

Collagen is an attractive biomaterial which is the major organic component of the extracellular matrix in native bone[39] promotes cell adhesion,proliferation, and osteogenic differentiation of bone marrow stromal cells, in vitro.[40] It is suitable for the formation of 3D scaffolds with controlled porosity.[40] Its mechanical characteristics diminishes with increase in porosity.[41] As it has good cytocompatibility it is an excellent substrate for the proliferation of mesenchymal stem cells (MSCs)in vitro.[42] Collagen cannot

withstand excessive loads, because it gets squeezed or compressed, so it cannot be used in load bearing areas. It has been used as a vehicle for growth factors, but this field is still under research. The denatured form of collagen termed gelatin[43] enhances osteoblast adhesion,migration, and mineralization.[44]

Chitosan is a polysaccharide which is well known due to its appealing characteristics. It displays antibacterial and antifungal activities. It helps in rapid blood clot formation, has analgesic properties[45] and accelerates wound healing that minimize the risk of scaffold contamination and postoperative infections, thus preventing failure of the scaffold.

Alginate is another commonly used polysaccharide, used in Bone Tissue Engineering (BTE) and regenerative medicine. It can be processable into different scaffold types[46] and encapsulates living cells.[46] Even though both alginate and chitosan do not exist within the human body, they show structural similarities to glycosaminoglycans (GAGs) of human tissues such as bone.

Some of limitations of these natural polymers are lack of bioactivity, has weak mechanical characteristics and somewhat rapid degradation rate[46] through enzymatic reaction. In order to overcome these limitations these natural scaffolds are combined with bioactive materials like bioceramics or mechanically strong ones e.g. synthetic polymers or metals

Biodegradable Synthetic Polymers.

The mostly used biomaterials of this category are aliphatic polyesters; they are polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA) and their copolymer poly(lactic-co-glycolic) acid (PLGA). They are of low cost and can be formed in large quantities. The shelf life of these biomaterial is long compared to natural polymers.[47]

Among this Polycaprolactone (PCL) is the most popular due to its biocompatibility,[48] mechanical stability, suitability for various scaffold fabrication techniques,[49] and remarkably slow degradation rate. Except PGA all others i.e. PCL, PLA and PLGA are hydrophobic.[50] Most of the available polyesters degrade by hydrolysis within the interior part of the biomaterial, with an empty shell formation, while the size is maintained for a considerable amount of time.[51] Because of this feature these biomaterials are mostly used as a bone graft substitute rather than drug-delivery purposes. They are moldable into the required shapes and have good mechanical properties even though these materials produce the acidic byproducts and has lack of bioactivity. Intracellular degradation of an acid can induce an inflammatory response.[52,53] In order to reduce this inflammation, hybrid scaffolds combining PLA and PGA with Calcium phosphate & Bioactive glass[54,55] have been created. So they are never used individually, but only as a combination. When used in bone regeneration, they are combined with growth factors and MSCs to obtain good results. Hydrogels, microspheres, blocks, and fibers are different forms fabricated from this biomaterial.[52]

Metals

Since metallic biomaterials show excellent mechanical properties[57,58] like they display high strength, toughness, and hardness, in comparison to polymers they are used in dental and orthopaedic fields to support the replacement of lost bone structure.[59] These metals enhance the mechanical properties of a scaffold by decreasing the pore size.[60]

Titanium and titanium alloys are encouraged in bone regeneration because of their high biocompatibility, appropriate mechanical properties and elasticity.[61] Nonetheless, lack of biodegradability of titanium and titanium alloys is a major disadvantage and might discourage their applications in bone regeneration. It also compromises patient satisfaction and increase health care costs as it needs second surgery for removal.[57]

Since last 10 years magnesium has been using because they have mechanical properties close to native bone and are completely biodegradable. Although they degrade by corrosion,[62] their byproducts are biocompatible. They are osteoconductive, increase the expression of osteogenic markers in vitro.[63] Utilization of magnesium alloys[64] or by coating pure magnesium with titanium[65] or ceramics[66] helps to overcome rapid rate degradation. But these metals lack bioactivity.

Composite Scaffolds.

When two or more different biomaterials combine to produce a "synergistic effect"in the overall resulting properties[67] and improve the mechanical, biological and degradation kinetics of a scaffold, they

are referred to as "composite" or "hybrid" scaffold. They are called "ternary"scaffold whenever[3] biomaterials are incorporated. They are used for Bone Tissue Engineering applications. And are divided into "polymer/ceramic," "ceramic/metal," and "polymer/metal." Composite scaffolds are obtained when PLA is enriched with dicalcium phosphate[54] or PGA and PLGA are combined with Hydroxyapatite (HAp) or β-Tricalcium phosphate (β-TCP) which increases the degradation time and improve the mechanical properties of the scaffolds.[40] Scaffolds containing HAp reinforced with collagen stimulate the differentiation of stromal cells in vitro and in vivo.[68] Collagen is also enriched with growth factors to induce osteogenesis or associated with MSCs and polypeptides in order to improve cellular colonization.[42] Others are PCL and bioactive glass coated with magnesium to implement bioactivity.[69]

Bioceramics.

Bioceramics are inorganic biomaterials. Among this Calcium phosphate bioceramics and bioactive are frequently used.[70] Bioceramics have unlimited availability, bioactivity, excellent biocompatibility, hydrophilicity, osteoconductivity and similarity to native bone inorganic components. So it is one of the effective biomaterial. Calcium phosphate bioceramics enclose hydroxyapatite (HAp), tricalciumphosphate (α-TCP and β-TCP) and biphasic calcium phosphate (BCP). They are paste like, can be moldable and easy to handle. It allow intimate adaptation to complex defects, which is difficult to accomplish with conventional bone grafting materials.[71]

It has the ability to form a strong bone-calcium phosphate bond[72] and has faster degradation rate. When tricalcium phosphate is combined with HAp, a mixture termed biphasic calcium phosphate (BCP) is produced.[73] BCP has significant advantages like stability,controlled bioactivity while promoting bone in growth especially in large bone defects,[74] and controllable degradation rate.[75] It has a higher degradation rate than HAp, yet slower than that of β-TCP.[76]

Another bioceramic is bioactive glass (BG), which is silicon oxide with substituted calcium. The bioglass used in intraoral applications (termed 45S5 Bioglass)[77] has a very slow degradation rate as it converts to a HAp-like material in the physiologic environment.[77] Typically, bioceramics degrade via multiple mechanisms.

It is very difficult to shape bioceramics into the desired structures because of their stiffness and low flexibility, moldability and its brittle nature.[79] They have weak mechanical strength[80] and fracture toughness,[5] so only used in non-load-bearing areas. The limitations of bioceramic scan be eliminated by combining it with synthetic polyesters or metals with are mechanically strong biomaterials and eliminate brittleness, difficulty in shaping, and weak mechanical strength.[81,82]

3D scaffold fabrication techniques

Different techniques are employed in the fabrication of 3D scaffolds because with the conventional technique heterogeneities in pore size, porosity, interconnectivity and architecture are unavoidable. Using conventional technique it is not possible for the fabrication of a custom-made scaffold with finely tuned architecture that replicates the complexity of native tissues and precisely conforms to the shape of a certain defect.

Three-dimensionally printed biomedical devices can precisely restore defects or potentially reconstruct entire organs with complex microstructure. Three-dimensional printing uses inkjet printing to apply a liquid binder solution onto a powder bed and can simultaneously arrange multiple cell types. Solid-free form fabrication (SFF) techniques, also known as rapid prototyping (RP), (Fig. 2) made possible to create scaffolds with precise external shape,internal morphology and "reproducible" three-dimensional architecture, despite their complexity.[83] Using either of the following technique; inkjet printing, laser assisted printing (e.g., Selective Laser Sintering (SLS), Stereolithography (SLA))and extrusion printing (e.g., fused deposition modeling (FDM))[84] they build complex structures layer by layer by "3D printing." These technologies represent additive manufacturing. Each printing method can be used for specific biomaterials and differs in resolution. Laser-assisted methods are used for biomaterials with wide range viscosities.[85] While extrusion printing is restricted to thermoplastic biomaterials such as PCL.[84] Inkjet, laser-assisted and extrusion-based techniques are used in printing of living cells and constructs.[84]

Initially a computed tomography (CT) scan of the patient specific bone defect is taken. Based on images from CT scans computer-aided design (CAD) models are produced. Then using computer-aided design (CAD) and computer-assisted manufacturing (CAM) technologies 3D-print the desired structure based on a CAD file that has already defined the exact dimensions of a scaffold.[86] This approach can be used in fabricating constructs that conform to a specific anatomical shape in a typical clinical case scenario. It provides promising results in preclinical investigations inperiodontal regeneration. There are few studies focused on subtractive technology (milling of a commercially available block, dictated by CAD/CAM technologies), which might not be sophisticated due to the lack of layer-by-layer addition.

Even though RP techniques are capable of producing constructs with satisfying mechanical strength it can be limited by the machine's resolution and material repertoire. A combination of RP techniques with different fabrication methods such as electrospinning[88] has been proposed because of the lack of sufficient resolution to fabricate nano and submicrometer structures.

Applications of 3D scaffold

When used in different periodontal applications: guided bone regeneration (GBR), guided tissue regeneration (GTR), vertical bone augmentation, sinus augmentation and socket preservation, it showed successful outcome. PCL has been the most utilized biomaterial. "Custom-made" 3D-printed PCL scaffolds based on medical imaging could show very effective results by permitting precise adaptation to the bony defect. In preclinical studies on rats fiber-guiding scaffold model in GTR was successful.[87] Another biomaterial is bioceramics that has been widely tested as part of 3D scaffolds for periodontal applications mainly in sinus and bone augmentation procedures. It is difficult to determine Ideal percentage of biomaterials to eliminate the risk of adverse effects for clinical uses. Natural polymers must be combined with mechanically strong materials. In GTR applications, the scaffold serves a dual role, as a grafting material and as a membrane. Since space maintenance is very essential for periodontal regeneration, it is necessary to utilize a mechanically strong scaffold. The combination of collagen with hydroxyapatiteis encouraged in bone tissue regeneration[89] due to the compositional similarities to native tissue combined with reasonable degradation rates for clinical uses.

Recent advances

In an animal study by Yun et al reported that non-woven PGA scaffolds give a potent structural support to stimulate the production of extracellular matrix in PDL cells.[90] So, PDL cells combined with PGA scaffolds aid in complex periodontal tissue regeneration.

Falguni et al described ornamenting 3D printed scaffolds with cell-laid extracellular matrix (ECM) for bone tissue regeneration.[91] The ECM-ornamented 3D printed scaffolds promoted osteoblastic differentiation of newly-seeded human nasal inferior turbinate tissue-derived mesenchymal stromal cells (hTMSCs) by increasing the level of four typical osteoblastic genes [4-fold higher osteocalcin; 4-fold higher osteopontin; 3-fold higher alkaline phosphatase (ALP); 4-fold higher Runt-related transcription factor 2 (RUNX2)] and increasing calcium deposition compared to bare 3D printed scaffolds.

In periodontal tissue regeneration a number of injectable biomaterials have been tested. Ji QX et al mixed quaternized chitosan with α-β glycerophosphate as an injectable carrier to deliver drugs and PDLSCs. When it was combined with basic fibroblast growth factors, it effectively induces new periodontal tissues in dogs.[92]

Another advance is the emergence of Three-Dimensional Printed Multiphase Scaffolds.[93] It is an approach for the regeneration of multiphase periodontal tissues by spatiotemporal delivery of multiple proteins. A progenitor cell / single stem population can differentiate into putative dentin/cementum, PDL and alveolar bone complex by spatially released bioactive cues and scaffold's biophysical properties. By using cell sheet technology in combination with additive 3D printing Lee et al developed a triphasic scaffold as an extension of biphasic scaffolds. The scaffold was fabricated by using fused deposition modeling. It has compartments for the cementum/dentin interface, the alveolar bone and the periodontal ligament.

Modern 3D printing technique uses multiple print heads which are loaded with different cell lines

and can fabricate complex multicellular tissue/organ. A dual 3D bioprinting technique was used by Cui et al to fabricate bone grafts which are large and functional with organized vascular networks.[94] Living tissues/organs, including bone and cartilage were fabricated by many researchers using diverse approaches with different biomaterials.[95]

Liu et al suggests that the prostaglandin E2 (PGE2), one of important inflammatory factor serves as an intriguing target for promoting regeneration of bone through modulating both inflammation and osteogenesis.[96] In biomimetric 3D nanofibrous scaffold PGE2 modulates bone morphogenetic protein-2 induced osteogenic differentiation.

Kazem-Arki M et al investigated the osteogenic effects of platelet rich plasma (PRP) coated nanofibrous Polyether sulfone / Poly (vinyl alcohol) (PES/PVA) scaffolds on adipose-derived mesenchymal stem cells and revealed that cells cultured on PRP coated PES/PVA scaffolds had the highest osteogenic differentiation.[97]

Patient Specific Scaffold modeling by computer-aided design software increases the architectural complexity. Rasperini et al in a case report used cone beam computed tomography (CBCT) scans of a patient's peri-osseous defect for designing a customized polycaprolactone scaffold fabricated through selective laser sintering.[98] The scaffold had an internal region with pegs to support PDL regeneration and the delivery of rhPDGF-BB. (Fig. 4)

The specificity of scaffold architecture is the ability to act as a biomaterial template for the guidance of oriented fiber formation which is critical to the regeneration of tissues. Fiber guiding can be achieved either by mechanically stimulating the cells before implantation or topographically guiding the orientation of fiber in order to accelerate functional attachment of the periodontal ligament. The initial approach utilized grooves of varying widths for inducing a spontaneous alignment or a specific topographical organization as initially demonstrated by Park et al.[99]

In a rat femoral bone defect when a porous scaffold entirely constituted of fibrinogen (Fg-3D) were implanted and investigated at two important time points. At 6 days post-implantation, Fg-3D led to early infiltration of granulation tissue and bone defect closure. At 8 weeks post-injury periosteum repair took place and Fg-3D led to reduced plasma levels of IL-1β and increased TGF-β1. So Fg-3D scaffolds can be considered immunomodulatory biomaterials.[100]

Future directions

Future approaches will need to consider the following key elements:

1) Occlusal load/biomechanical influences of the regenerated tissues.

2) Effects of microbial load and contamination of wounds.

3) Wound stability to maintain the 3D conformation of the wound site.

4) Appropriate cellular signals to recruit and direct cell populations.

Ongoing research in nanotechnology for regeneration of periodontal complex tissues will further elucidate the required scaffold design parameters and therapeutic capabilities of nanotechnology-based application. Future approaches in these new technologies should work toward a common goal: to make available cost-effective, patient-specific treatment options that provide maximal function and esthetics.

Conclusions

Scaffolding matrices can be considered as an attractive alternative to bone replacement grafts in endosseous implant placement. Periodontal attachment may be the key to preserving and supporting natural teeth. Various techniques are developed for the purpose of periodontal tooth supportive complex regeneration. Furthermore,these promising advances provides the opportunity for huge paradigm shifts in dental tissue engineering from replacement to regeneration. So, the success and the future of periodontal regeneration depends upon our understanding and ability to recognize those clinical situations that will benefit from one or more of these new emerging technologies.

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JSPIK

Management of gingival recession through lateral pedicle graft using iso amyl 2-cyanoacrylate

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ABSTRACT

Gingival recession may result in sensitivity, unaesthetic appearance and root caries. Root coverage procedures are generally performed to rectify this defect. The challenge in root coverage is to 'secure' the flap on the denuded surface so as to eliminate dead space. Sutures had been used through ages to attain this purpose. Isoamyl 2-cyanoacrylate is a tissue adhesive material which could be used as an alternative for sutures. This article highlights the root coverage procedure using lateral pedicle graft which is secured using Isoamyl 2-cyanoacrylate as an alternative to sutures.

Key words: Gingival recession; Isoamyl 2-cyanoacrylate; Tissue adhesive; Root coverage

Introduction

Gingival recession is the exposure of root surface by an apical shift in the position of the gingiva.[1] Root coverage procedures aim to restore the gingival margin coronal to the CEJ along with periodontal regeneration. Various techniques such as free gingival graft, lateral pedicle graft, connective tissue graft, coronally displaced flap and double papilla have produced predictable root coverage. Of these the lateral pedicle graft has been a commonly used root coverage procedure when the adjacent tooth has adequate attached gingiva. Not only the technique, but also proper wound closure is necessary for a positive result. Braided silk has a phenomenon of "wicking," making it a site for secondary infection.[2] Furthermore, it has the maximum amount of inflammatory tissue response (Postlethwaite 1974). Hence, a need for an alternative to sutures is felt. Cyanoacrylate is a tissue adhesive that was discovered in 1959 by Coover et al.[3] This case report portrays the efficiency of Iso amyl 2-cyanoacrylate as a bioadhesive in root coverage procedure done using lateral pedicle graft.

Case report

A patient named Mrs. Kavitha, 30 years old female came to our college with the chief complaint of receding gums in the lower front tooth region for the past 6 months and wanted it to be treated. The patient was referred to the Department of Periodontology and Oral Implantology for management. On intraoral examination 41 was found to be buccally placed with Miller's class I gingival recession (Fig 1). Patient's medical and dental histories were non-contributory. Scaling and root planing was performed and the patient was recalled after 4 weeks for evaluation. Root coverage procedure in 41 was planned. The width of attached gingiva on the adjacent tooth 42 was found to be adequate. So lateral pedicle graft placement was planned along with Isoamyl 2-cyanoacrylate to secure the flap. The procedure was explained to the patient and written consent was obtained from the patient.

Surgical procedure

The surgical site was properly isolated and anaesthetised. Root planing was performed in 41. De-

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epithelization of the area adjacent to the recession site was done to prepare the recipient site. A crevicular incision was given using #15 BP blade involving the distal line angle of 42 and the mesial line angle of 43 involving the interdental papilla. Vertical incision was then given from the distal line angle of 42 and the mesial line angle of 43 extending beyond the mucogingival junction, with the flap wider at the base for sufficient vascularity. Adequate mobility of the flap was ensured by extending the vertical incision more apically. The flap was then reflected using a periosteal elevator and slid laterally onto the adjacent denuded root. The flap was adapted properly without excess tension (Fig 2). 0.5ml of Isoamyl 2-cyanoacrylate (Fig 3,4) was loaded in a syringe and ejected along the margins of the flap (Fig 5) so as to secure the flap on the recipient site. A periodontal dressing was given to protect the surgical site. (Fig 6)

Postoperative instructions

Analgesics were prescribed twice daily for three days for postoperative pain. 0.2% chlorhexidinedigluconate was also prescribed to be used twice daily for 3 weeks. The patient was asked to refrain from tooth brushing at the surgical site for 2 weeks.

Result

7 days after surgery, the periodontal dressing was removed. The wound healing was not complete (Fig 7). So the site was irrigated with normal saline and repacked with periodontal dressing. The dressing was removed 7 days later. Healing was uneventful and there was good amount of attached gingiva with complete root coverage on clinical examination after two weeks. (Fig 8). Post operative observation after three months shows increase in the width of attached gingiva with a mild apical displacement owing to the position (buccally placed) of 41 (Fig 9).

Discussion

The aim of all wound closure techniques is to approximate the edges of the wound so that uneventful healing takes place naturally. In wound closure the primary focus is normally on relieving tension on the wound and bringing the tissue edges together. Precise approximation of the incised margins is critical for favorable esthetic and functional results. The most commonly used method for attaining wound closure is suturing. However it has several disadvantages like requirement of anesthesia, greatest tissue reactivity, higher cost, more time consuming, need for suture removal, and associated risk of needle stick injury.[4]

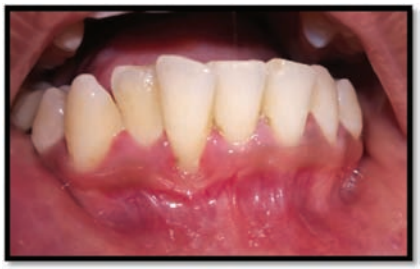

Figure 1: preoperative

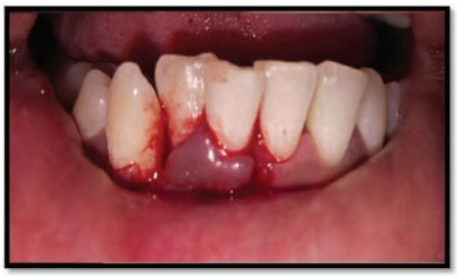

Figure 2: Flap reflected from 42 and laterally repositioned on 41

Figure 3: Iso amyl 2-cyanoacylate

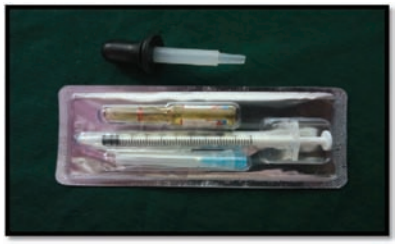

Figure 4: Iso amyl 2-cyanoacylate

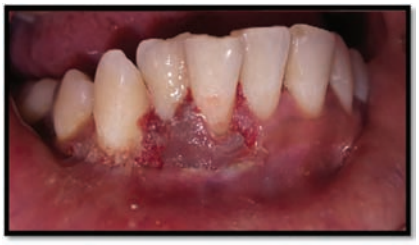

Figure 5: Cyanoacrylate placed

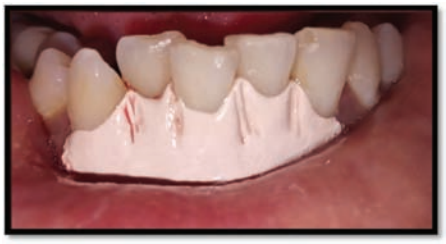

Figure 6: Periodontal dressing given

Because of the complications of suturing, alternative techniques like staples, adhesive tapes and tissue adhesive have emerged to attain a better wound closure. Tissue adhesives prevent tearing of the wound margin while closing the wounds even in patients having thin gingival biotype. Clinical use of cyanoacrylate was approved at the beginning of 1996. Cyanoacrylates include short chain (methyl and ethyl cyanoacrylates) and longer chain (butyl, isobutyl, isoamyl, and octyl cyanoacrylates) derivatives.[5]

Padhye and Pol stated that cyanoacrylate has an added advantage with respect to time and methodology of diminishing the two-step procedure of suturing followed by periodontal dressing to just one step of application of the material.[6]

Time taken during surgical procedures using cyanoacrylate is crucial because it:

- Lessens trauma to the patient
- Lessens fatigue to the surgeon
- Reduces postoperative swelling and operating time
- Easier to apply than suture
- More comfortable to the patient.

Advantages of cyanoacrylate from subjects point of view: [3]

- At times, removal of sutures is annoying/ painful for some patients
- There is more discomfort on sutured site in the days after surgery.

Forrest reported that the tissue adaptation is better as it provides fixation of the flap to the whole surface, while sutures provide only marginal fixation.[7]

Use of cyanoacrylate provides better tissue adaptation and faster healing. Closure of wounds without the need for sutures is a major advancement and an opportunity to reduce pain and anxiety caused by the treatment.[8]

In the given case, since the patient was not willing for orthodontic management, root coverage procedure was the only option. Iso amyl 2-cyanoacrylate was used for achieving wound closure in treating gingival recession in buccally placed 41 using laterally positioned graft. Even though the case selection was not ideal, the outcome was positive and patient satisfaction was immense. Primary closure was obtained

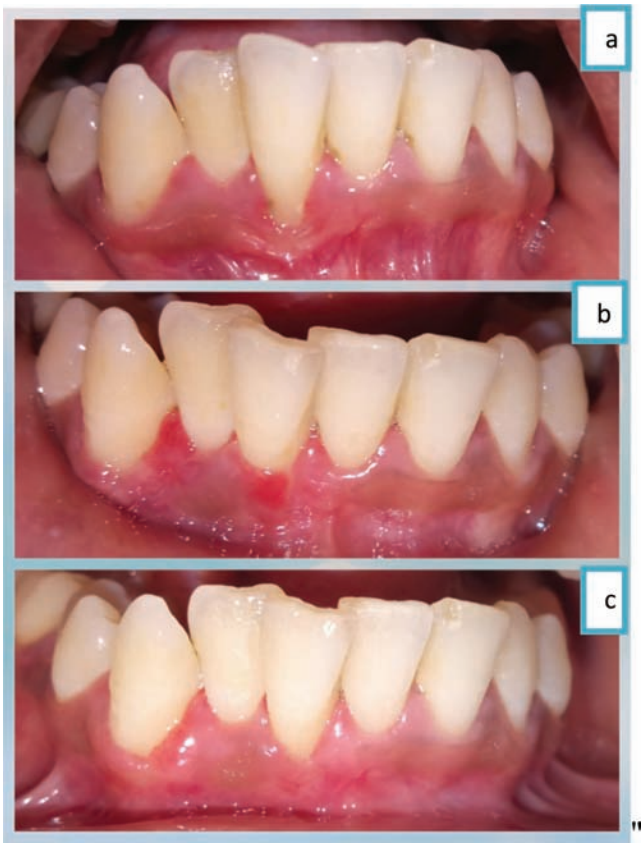

Figure 10: (a)pre-op; (b)2 weeks post-op; (c)3 months post-op

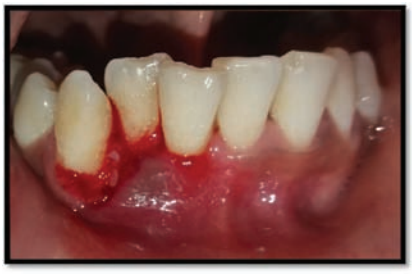

Figure 7: 1 week post-op

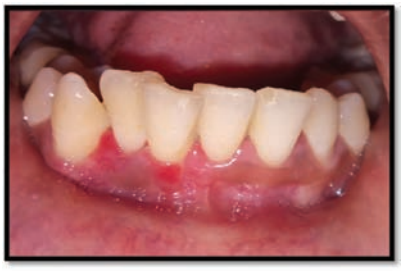

Figure 8: 2 weeks post-op

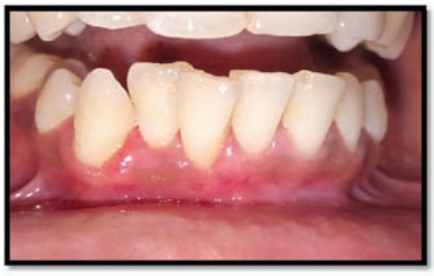

Figure 9: 3 months post-op

at the donor site and the healing was uneventful. The patient was referred to the Department of Orthodontics for further management.

Conclusion

The dual need of both patient comfort and better results has brought about various changes in the traditional surgical procedures. The use of Iso amyl 2-cyanoacrylate as an alternative to traditional sutures can be considered as one such change. Root coverage is a procedure where the sole purpose is to replace the flap to the desired position to increase the width of attached gingiva. The efficiency of cyanoacylate can be best demonstrated in such a procedure. It is concluded that Iso amyl 2-cyanoacylate can be used as an alternative for sutures yielding similar results.

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Interdisciplinary orthodontics: exploring the benefits of PAOO in anterior alveolar augmentation and accelerated tooth movement

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ABSTRACT

Periodontally accelerated osteogenic orthodontics (PAOO) is a relatively new procedure designed to minimize the time taken for orthodontic treatment. The orthodontist avails of the aid of a periodontist to perform decortication of the bone and places bone graft for rapid orthodontic correction of malocclusion. PAOO can play an important role in the comprehensive treatment of a patient's occlusal and esthetic needs. This technique has been shown to increase alveolar bone thickness, decrease treatment time and enhance post treatment orthodontic stability.

Keywords: Periodontally accelerated osteogenic orthodontics; decortication; orthodontic stability

Introduction

The correction of irregular teeth can take a prolonged period of time, depending upon various factors such as good patient compliance for obtaining optimal results, treatment mechanics etc.[1] The search for efficiency, especially in decreasing the treatment duration without compromising the optimum results has become a preliminary goal in all areas of orthodontics. Various treatment modalities such as low friction self-ligating bracket systems, robot preformed archwires, rapid canine retraction have been attempted as a means of reducing the treatment duration.[2]

Periodontally accelerated osteogenic orthodontics also known as wilckodontics is a combination of selective decortications and alveolar augmentation.[3] The technique described provides a net increase in alveolar volume after the orthodontic treatment.[4]

The technique is claimed to have many advantages such as reduced treatment duration, enhanced expansion, differential tooth movement, increased traction of impacted teeth and orthodontic stablility.[5] The enhanced tooth movement is mainly due to the surgical wounding of cortical bone which leads to the production of a burst of localised hard tissue remodelling which is described as regional accelerated bone remodelling phenomenon.[6] As osteoclastic activity is a key element in the kinetics of tooth movement, the induced osteopenia makes bone more pliable to orthodontic forces.[5]

History:

Corticotomy facilitated tooth movement was first described by LC Bryan in 1893.[5] However it was first introduced by Kole as a means of rapid tooth movement.[7] A more recent surgical orthodontic therapy was introduced which involved the combined strategy of corticotomy with alveolar grafting, termed as accelerated osteogenic orthodontics and more recently to as PAOO. Several reports claim this technique to be safe, effective, extremely predictable and associated with less root resorption and reduced treatment time and a reduction in the need for or-

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thognathic surgery.[5]

Rationale for using PAOO:

1. Provides a four to five month window of opportunity providing enough time to accomplish tooth movements.

2. As it creates an increased differential between anchorage and activated teeth, it increases the movement of the activated teeth against the non-activated teeth thus augmenting anchorage.[3]

Biology underlying PAOO:

In this technique the cortical bone is scarred surgically to be moved followed by grafting. The patient is seen every two weeks and the rapid tooth movement produced after PAOO is substantially different than periodontal ligament mediated tooth movement.[5]

A localised osteoporotic stage as part of the healing event known as regional acceleratory phenomenon could be responsible for the rapid tooth movement after PAOO.[8] RAP was first described by Frost in 1983,though this phenomenon has been familiar to many histomorphometrists since 1966. He noted that the original injury accelerated the normal healing process. This acceleration is Regional Acceleratory Phenomenon (RAP).[5]

Use of Proline Rich Protein:

Platelet rich plasma (PRP) is an approach in tissue regeneration which has been widely used in various surgical fields including dental surgical procedures. PRP has become a valuable adjunct to promote healing in many procedures in dental and oral surgery including ablative surgical procedures, and surgical repair of the alveolar cleft and periodontal plastic surgery. In such procedures, the adhesive nature of PRP facilitates the easier handling of graft material, with more predictable flap adaptation and hemostasis. It also provides a more predictable seal than is the case with primary closure alone" [9]

Due to these factors, platelet rich plasma was placed along with the graft material for the patient whose case report has been presented in the article.

PRP Preparation:

A volume of 60 ml of whole blood was drawn from the medial cubital vein of the patient using three 30 ml syringes that each contained 3 ml of 10% sodium citrate solution as an anticoagulant. The PRP used in the procedure involved the use of machine centrifugation process which was sterile and precisely suited to platelet separation from RBC and their sequestration in high concentrations without lysing the

Fig 1.PRP separated after centrifugation

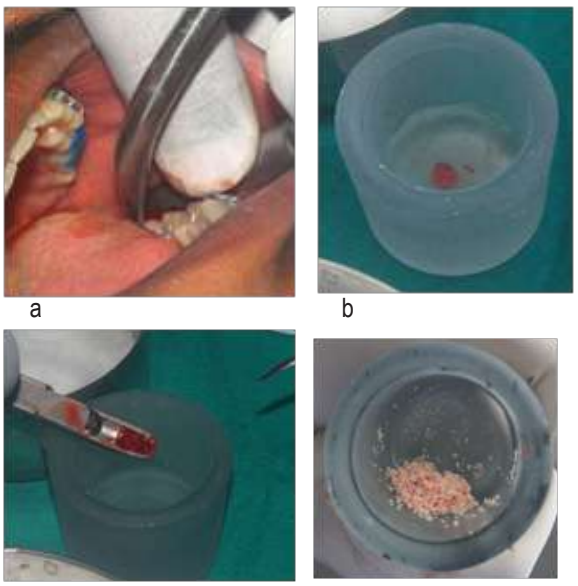


Fig 2.a,b,c bone graft obtained from extraction site d. autograft mixed with PRP and allogenous graft in a mortar

platelets or damaging them from losing their secretory potential.[10]

Preparation of autogenous graft

Autogenous bone graft that was used in the technique was harvested from the extraction site in the mandibular third molar region. Particulate bone was harvested with bone scrapers and mixed with PRP.

The uniqueness of this case report was that autogenous graft mixed with PRP of the patient was placed in the corticotomy site to enhance the bone density in the anterior alveolar region. A small amount of allogenous graft was also mixed though it was not the predominant ingredient.

Case report

A 22 year old male patient reported to the Department Of Orthodontics complaining of forwardly placed lower front tooth. He wanted to complete his orthodontic treatment faster than the normal treatment time of 2 years.

The patient presented the following malocclusions:

1. Class III molar and canine relation bilaterally
2. Reverse overjet -1mm
3. Overbite – 0.5mm
4. Spacing in upper and lower dentition
5. Concave profile

He was not willing for the traditional treatment options given to him such as bilateral sagittal split osteotomy or enmasse distalisation of the lower dentition using mini implants. A decision was made to perform PAOO.

Rationale:

1 To accelerate orthodontic tooth movement

2 To augment bone support in the lower anterior region (class III patients are prone to have thin labial cortical bone)

Pre treatment intraoral photographs

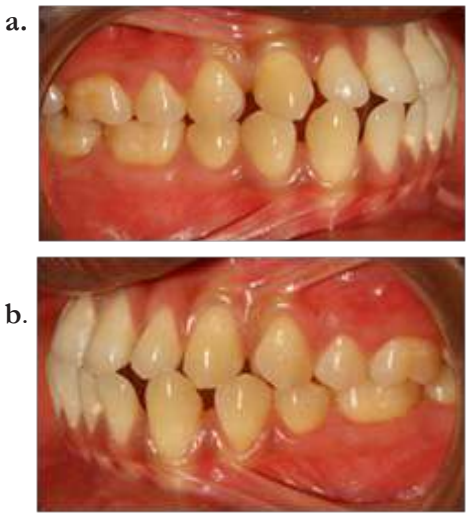


Fig. 3 a, b Class III molar and canine relation bilaterally

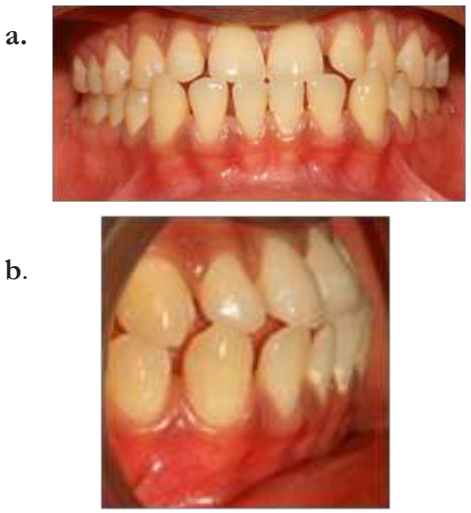


Fig.4 a,b Washboard appearance of lower anteriors

Mid treatment photographs prior to surgery

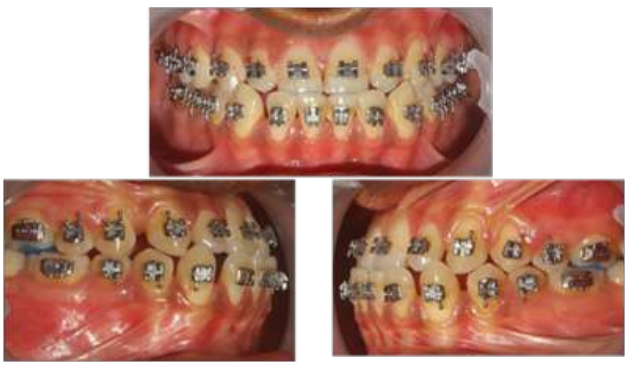
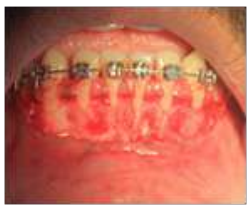
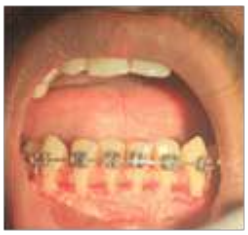


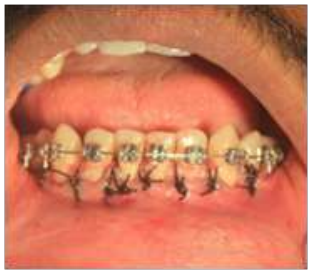
Fig.5

Surgical procedure

a.







c.

Fig 6. Placement of a mix of autogenous and allogenous bone graft along with platelet rich plasma at the site

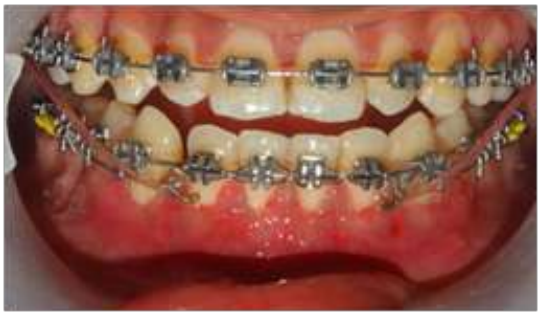
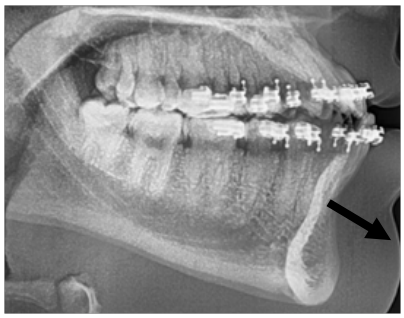


Fig.7, 8. One Week After Surgery



New bone formation

Fig.9.Pre Treatment

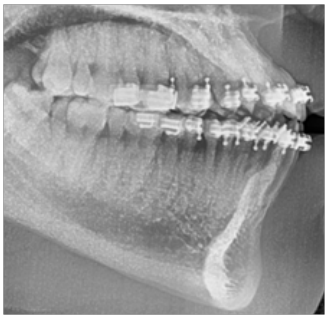


Fig.10.Post Treatment

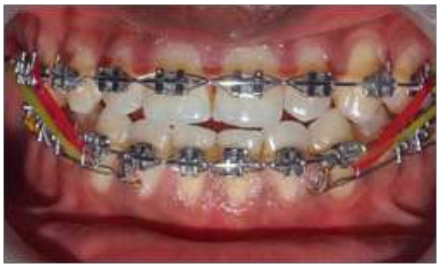


Fig.11. Retraction Begun

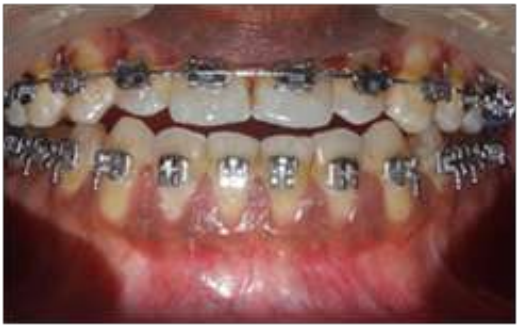


Fig.12. After two Months of Treatment

Surgical procedures

All surgical procedures were performed after obtaining the consent of the patient. Under local anesthesia, full-thickness envelope flaps were raised with sulcular incisions while preserving interdental papilla on the buccal sides of maxillary and mandibular anterior regions only. No flap elevation orcorticotomy was performed on the palatal or lingual side in this case. Vertical corticotomy cuts were performed with round burs (size 2) with saline water irrigation and in between roots from the distal of canines to the distal of the opposing canine in the lower arch. These vertical cuts were extended approximately 2 mm past the apices of the teeth. The vertical corticotomy cuts stopped about 2 mm short of the alveolar crests. Both corticotomy cuts and perforations were extended through the entire thickness of the cortical plate, just barely into the cancellous bone. Particulate bone was laid down on the mandibular anterior region.

The mucoperiosteal flaps were replaced and sutured. The patient was given amoxicillin, 500 mg t.i.d. for 5 days and chlorhexidine mouth rinse 0.12% b.i.d. for 2 weeks. Analgesics was prescribed for postoperative discomfort. The sutures were removed after 1 week.

Orthodontic procedures

Levelling and alignment of the upper and lower arches had been completed and proceeded to retraction stage before the surgery. Active treatment with class III elastics was begun within a week.

Discussion

Several studies have demonstrated a correlation between facial type and alveolar bone morphology of the mandible. In 1996, Handelman[11] et al. showed that vertical growth strongly correlated with alveolar bone thickness, with low mandibular plane angle cases displaying thicker bone lingual to maxillary and mandibular incisors and high mandibular angle cases displaying thinner bone labial to the mandibular incisors. There appeared to be a direct relationship between increased facial and alveolar height and thinness of the alveolar bone, presumably because the incisors continue to erupt to maintain overbite, and the alveolus becomes attenuated with thinning of the width between the labial and lingual walls.

In 2007, Yamada et al., [12] using computed tomography, found that thin alveolar bone anteroposteriorly was associated with high mandibular plane angles and class III malocclusions.

Facial tipping of incisors can result in thinning of facial alveolar bone. In this case, the thickness of mandibular buccal plates was already compromised leading to a washboard appearance in the lower anterior region which further accentuated with fixed orthodontic treatment which could lead to undesirable sequalae such fenestrations and dehiscence.

The traditional technique described by Wilcko and Kole was not followed in this procedure.

Modifications

1. The corticotomy cuts were made only on the facial cortical bone

2. Autogenous bone grafts mixed with platelet rich plasma obtained from the patient was used

Two months after surgery the periodontium in the lower anterior region seems to be improved and better supported when compared the pretreatment condition. The rate of incisor retraction was also noted to be increased and a positive overjet was achieved within 2 months.

Conclusion

As the article presents a single case report, the conclusions reached are limited. More clinical studies with an increased sample size is recommended.

However, this case suggests the following conclusions:

1. PAOO is an effective treatment approach in adults to accelerate the treatment and reduce the risk of root resorption.

2. The utilisation of a modified surgical approach that limited the corticotomy procedure to the buccal/labial cortical bone produced the required RAP thus providing a good alternative to the conventional technique that involved both the buccal and lingual bone.

3. The reduction in the time required for surgery and decreased patient discomfort are basic advantages to a modified surgical approach.

4. More clinical research is needed to determine the optimal amount of autogenous bone graft.

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Evaluation of antimicrobial activity of Punicagranatum against Porphyromonasgingivalis: An Invitro microbial study

ArthiieThangavelu[1], Rajasekar Sundaram[2], Tamilselvan Kumar[3], Sivakumar Annamalai[4]

Introduction:

Periodontitis is an inflammatory condition which involves both direct tissue damage resulting from plaque bacterial products, and indirect damage through bacterial induction of the host inflammatory and immune responses.[1] The mechanical debridement such as Scaling and Root Planing (SRP) reduces the level of subgingival bacteria but does not completely eliminate the pathogens that resides deep into the connective tissue.[2] The limitations of conventional periodontal therapy led to the emergence of many chemical agents such as antiseptics, NSAIDS and antibiotics.[3] In order to overcome the drawbacks of systemic administration such as bacterial resistance, drug interactions and inconsistent patient compliance, local drug delivery system was introduced.

In the Local Drug Delivery systems, the drug can be delivered to the site of disease activity at a required concentration and can facilitate prolonged drug delivery. Due to the various side effects of chemical products, natural phytochemicals isolated from plants are considered as good alternatives. [4] Among the many herbs available, Pomegranate extracts have been reported to have many beneficial health effects, exhibiting antibacterial, antioxidant, anti-inflammation, antiproliferative, and DNA repair activities.[5]

Punica granatum (pomegranate) belongs to the Punicaceae family, and is rich in polyphenols such as Ellagitanins, Punicalagins, Punicallin and Gallotanin. The antibacterial effect is due to the tannins, which increases bacteriolysis and interferes with bacterial adherence onto the tooth surfaces.[6] Apart from the anti-bacterial action, pomegranate extract exhibited anti inflammatory activity through the inhibition of NF-kB activity and prevention of ERK-1 and ERK-2 activation.[7] Pomegranate extract have been shown to inhibit the IL-1β induced destruction of proteoglycan, expression of MMPs at the cellular level, NO and PGE2 production.[8] Pomegranate thereby could be an excellent adjunct to the conventional periodontal therapy as an anti-plaque agent due to its antibacterial, anti-oxidant and ani-inflammatory properties. Therefore, the present study was designed to evaluate the anti-microbial effect of pomegranate containing film in-vitro on reference strain of Porphyromonas gingivalis.

Materials and methods:

Preparation of standard bacterial suspensions:

The average number of viable, gram negative Porphyromonas gingivalis organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (10^5-10^6) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Antibacterial Activity Screening:

Determination of the Minimal Inhibitory Concentrations (MIC):

MIC was determined with 96-well plate

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microdilution method. The bacterial strain was grown for 24 hours anaerobically and inoculated into a final volume of 100 µL of new half- strength BHI broth containing 2-fold serial dilutions of samples. The final optical density of the bacterial cells was adjusted to 0.1 at 600 nm in 100 µL of mixture. The mixture was cultured anaerobically at 37 °C for 48 hr and the bacterial growth was evaluated via measurement of the optical density at 600 nm. The lowest concentration at which no growth (OD600nm ≤ 0.1) observed was defined as MIC (µg/mL). The concentration of the sample used for MIC were between 100-7.25 µg/mL.

Preparation of PC periodontal films:

Punicalagin (PC), was obtained from Carbosynth Ltd, United Kingdom. (Fig 1) The percentage of purity of PC was assessed by HPLC and was found to be > 98%. Film formulations were prepared using the solvent casting technique. (Tabe 1) Gelatin solution (20%) was prepared by dissolving gelatin in Millipore water. 1% v/v glycerol, which was used as a plasticizer to impart adequate flexibility to the produced films. The calculated amount of PC (10mg/film) was added to the final gel before casting. The pH of gel formulation was adjusted to about neutrality (pH 6) by the addition of triethanolamine (0.3%) before casting. The gels were casted into square cube trays and allowed to dry in a levelled oven maintained at 30 C, for a period of time enough to produce flexible, dry film with constant weight. The dried films were cut into rectangular (5X2 mm) patches, packed in aluminum foil and stored in desicator which was maintained at room temperature.

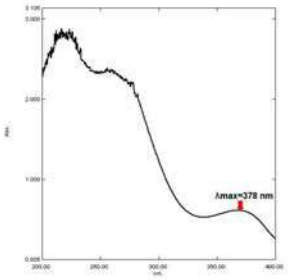
Physical characterization and content uniformity test:

Assessment of weight and thickness was done on six randomly chosen film patches from each formulation using a sensitive balance (Electronic balance, Sartorius AG, weighting technology, BL-210S, Germany) and a digital micrometer (Tricircle micrometer, China), respectively. Determinations were performed in triplicate. Drug content uniformity was tested on six randomly selected film patches of each formulation. Each drug-loaded patch

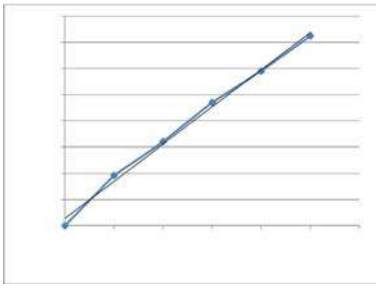
Figure 1: Punicalagin Powder

Figure 2: Punicalagin Gelatin Film

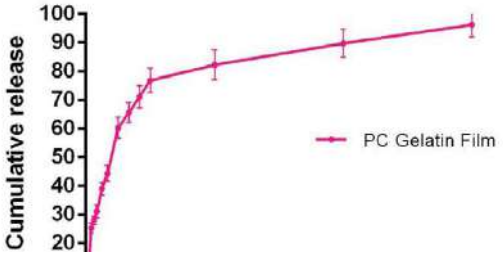
Figure 3: Cumulative Release Profile of Pc From Gelatin In



Graph 1: UV-vis spectra of punicalagin



Graph 2: UV-vis calibration curve of punicalagin



Graph 3: Cumulative release profile of pc from gelatin in

was allowed to dissolve in 100 ml of Sorenson's phosphate buffer pH 6.6. The concentration of PC in the patch was determined spectrophotometrically at 378 nm. UV standard curves were constructed over a concentration range of 5 - 25 µg/ml. All results are presented as mean ± standard deviation.

Surface pH: Film patches were allowed to swell for 2 hours on the surface of agar plates prepared in Sorenson's phosphate buffer pH 6.6. The surface pH was determined using pH paper placed on the surface of the swollen patch. A mean of three readings were recorded.

Determination of Release kinetics of PC film:

In all the films, gelatin was added at a concentration of 20% before casting to provide consistency and homogeneity to the gel mixtures. The concentration of gelatin was selected based on preliminary screening of different concentrations. The gelatin had supporting effects on the films mechanical properties and flexibility, which would be advantageous for periodontal applications. All films were cut equally into rectangular patches of diameter 5X2 mm (Fig 2). Physical characteristics and drug content of all formulations were determined. PC periodontal films patches ranged from 27.02 to 29.24 mg weight and from 1.8 to 2.2 mm thickness. The surface pH of all formulations was about neutrality as adjusted by the addition of triethanolamine before casting and ranged from 5.8 to 6. Percentage drug content indicated drug uniformity and distribution all over the prepared films as indicated by the relatively small values of standard deviations.

Table 1: Composition of pc periodontal films

Composition	Concentration (%)
PC	10mg/film
Gelatin	20
Glycerine	3
Triethanolamine	0.3

Each disc (5X2 mm) was weighed and placed into a 5 ml vial containing 2 ml Sorenson buffer of pH 6.6, previously warmed at 37^{0}C. The closed vials were placed in a thermostatically controlled water bath preset at 37^{0}C, until the end of the experiment. The whole volume was withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 6, 8, 24, 48 and 72 h) and replaced by fresh warmed buffer solution. The samples were assayed for PC spectrophotometrically at λmax 378 nm, and the cumulative drug concentrations were calculated (Graph 1). All experiments were done in triplicate and the values were presented as the mean ± standard deviation. Blank films were also subjected to the release study to detect the contribution of the polymers used.

Results:

Determination of MIC by micro-dilution method:

The test sample exhibited the strongest antibacterial effect on the tested strain with MIC of 12.5µg/mL for the P. gingivalis.

Calibration curve:

1mg of PC was transferred into a 10mL of volumetric flask and the volume was made up to the mark with mobile phase to give 100µg/mL stock solution. From this stock solution a series of dilutions ranging from 5 - 25 µg/ml was prepared. The sample was analysed through UV-Vis spectrophotometer at a λmax of 378 nm and the calibration curve was plotted (Graph 1 and 2).

In vitro release study:

A burst release of PC from both types of films was observed throughout the first 2 h (Fig 3). This effect was followed by a decrease in the release rate for the next 10 h, then by a marked decrease in rate to the end of the study. Release of PC from gelatin films was performed in 2 ml buffered system at pH 6.6 to simulate the small space available of the periodontal

TABLE 2: Physical characteristics of the prepared films

Formulation	Weight (mg±SD)	Thickness (mm ± SD)	Surface pH	Drug content (mg)
PC Gelatin film	28.47 ± 3.13	2.13 ± 0.012	5.8-6	9.6 ± 0.8

cavity. The general initial increase of the dissolution of PC from gelatin films could also be attributed to the presence of the water-soluble gelatin and also water-soluble hydrophilic additives in these films that would dissolve rapidly introducing porosity. The formed voids will in turn allow for the entrance of the release media and its diffusion through the film. (Graph 3)

Discussion:

The results of the invitro study showed that the PC gelatin film had an excellent anti-bacterial effect against Porphyromonas gingivalis. This was in accordance with the study by Bhadhade et.al[9] who showed that pomegranate mouthwash had antibacterial efficacy against Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi). The study done by Menezes et al,[10] showed that P. granatum exerts a significant activity against microorganisms commonly present in the dental plaque. Punica granatum had shown antimicrobial activity against Eikenella corrodens, which is a secondary colonizer in the biofilm formation more than chlorhexidine.[11]

Punicalagin causes disruption of cytoplasmic membrane and inhibition of sucrose digesting enzyme of microbes.[12] The extract of P. granatum showed strong antimicrobial activity on both gram-positive and gram-negative oral bacteria.[13] Pereira et al[14] examined a gel derived from P. granatum, inhibited the glucan synthesis and exerted antimicrobial action even on the already formed biofilm.

Additional to the antibacterial effect Punicalagin has an anti-inflammmatory and strong anti-oxidant actions. Thus our study concluded that Punicalagin film can be used as local drug delivery and hence a clinical trial has been designed to investigate the efficiency of Punicalagin film as an adjunct to scaling and root planing in chronic periodontitis patients.

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Effect of irradiation of 810nm laser on bone for 270 sec: A rabbit histological study

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ABSTRACT

Introduction and Objective: A biphasic response has been demonstrated many times in LLLT research and it suggests that if insufficient energy is applied there will be no response (because the minimum threshold has not been met), if more energy is applied the then a threshold is crossed and biostimulation is achieved but when too much energy is applied then the stimulation disappears and is replaced by bioinhibition instead. The aim of our study was to investigate the effect of GaAlAs 810nm diode laser under single irradiation for 270 sec on healing of bone.

Materials and Method: Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 8 months old for the study. Femur was chosen as site of surgery. The centre of the femur was drilled using implant osteotomy drills to the size of 2.8mm in width and 6mm in depth. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were wavelength of 810nm, 90mW, for 270 seconds in continuous mode using the non-initiated disposable fiber of 300µmm diameter in punctual contact. Contra lateral femur was used as a control and the laser was sham treated. At the end of 2 weeks samples were collected from the surgical area and slides were prepared and assessed histologically.

Results: At 14th day, the lased group showed areas of haemorrhage, abundant amount of inflammatory cells. There was no evidence of bone formation in lased site. Non-lased site showed adipose and lymphomatous tissue with no new bone formation.

Conclusion: The results of the present study using 810nm, 90mW, for 270 sec for single session inhibited the formation of collagen and bone formation in two weeks. So the above parameters can be regarded as inhibitory dose for formation of bone.

Introduction

The term "laser" comprises nothing but monochromatic light. The "light" is generally accepted to be electromagnetic radiation ranging from 1 nm to 1000 nm in wavelength. The visible laser spectrum ranges from approximately 400 to 700 nm. Lasers are divided into High-level laser therapy (HLLLT) and low-level laser therapy (LLLT).[1]

LLLT may enhance neovascularisation, promote angiogenesis and increase collagen synthesis to promote healing of acute[2] and chronic wounds[3]. LLLT can also stimulate healing of deeper structures such as nerves[4], tendons[5], cartilage[6]. LLLT can reduce pain[7], inflammation[8] and swelling[9] caused by injuries, degenerative diseases or autoimmune diseases. The exact mechanism of action of LLLT in biomodulation of bone healing is not known.

A biphasic response has been demonstrated many times in LLLT research[10,11]. The expected dose response to light differs at a subcellular, cellular, tis-

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sue or clinical level. It can be intrepeted that if more than required energy applied it may lead to bioinhibition and if insufficient energy is applied, then there will be no stimulation as the minimum threshold has not been met. So there exists an optimal window of energy as and when it is utilized then there will be biostimulation.[12] In-vitro studies showed that LLLT was effective in the proliferation of bone marrow stem cells, osteoblasts and osteoclasts.[13,14,15] On the other hand, Coombe[16] et al showed no effect of laser on osteoblastic cells. Gerbettaz[17] et al. showed no changes in the proliferation of bone marrow mesenchymal stem cells between lased and unlased groups. In spite of promising results obtained from in-vitro and animal studies, the biphasic dose response remains and different results for use of different choice of lasers, wavelengths, energy density, power density, the method of delivery and frequency of treatment. The single-use photo biomodulation therapy on healing of bone is not explored thoroughly. So, the study is undertaken to evaluate the use of low-level gallium aluminium arsenide (GaAlAs) diode laser on the healing of bone using 810nm wavelength, at the power of 90mW, using the fibre of 300µm with single irradiation for 270 sec.

Materials and Methods

The study was carried out following the guidelines of the CPCSEA and institutional ethical committee and obtaining their approval. Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 8 months old for the study. Before the surgery the animals were anesthetized using ketamine (15mg/Kg) and xylazine (10mg/Kg.). Antibiotic prophylaxis started prior to the surgery (ceftriaxone 500mg). Femur was chosen as site of surgery, the skin overlying the femur was shaved and disinfected with Povidone-Iodine solution. 3 cm incision was given upto the bone on the lateral aspect of the femur exposing the underlying fascia and bone. The muscles were retracted using surgical elevators. The centre of the femur was drilled using implant osteotomy drills and widened 0.8 mm under copious irrigation of normal saline. Final dimension of osteotomy site was 2.8mm in width and 6mm in depth.(Fig.1) The sites were cleaned with irrigation of saline. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were wavelength of 810nm, 90mW, for 270 seconds in continuous mode using the disposable fibre of 300µmm diameter. Multiple points were chosen for irradiation of laser along apex, mid-medial, and mid-lateral of osteotomy site. (Fig.2,3) Contralateral femur was used as a control and the laser was sham treated. After the irradiation, the surgical site was sutured in layers using catgut 2.0 (Fig.4). Post operative antibiotics (ceftriaxone 500mg) was continued twice daily IM for 5 days. They were kept in the animal house and were fed vegetable diet. At the end of 2 weeks all animal were euthanized using high dose of Thiopental sodium. Samples were collected from the surgical area and restored in 10% buffered formalin. After that specimens were subjected to 4 % EDTA for demineralization. Longitudinal cuts were made to divide the bone in to two halves. Then slides are prepared and stained with Haematoxylin and eosin (H.E. stain, Merck). All tissue specimens were examined by light microscopy and were assessed semi-quantitatively, on a graded scale, under a 10X magnification objective and a fixed grid[18]. Images were captured using compound microscope (Olympus).

Results

At 14th day, the lased group showed compartmentalized areas with collagen fibers, proliferating blood vessels, extravasated RBC's, delicate collagen fibers, proliferating blood vessels with mild to moderate dilatation, areas of haemorrhage, abundant amount of inflammatory cells. There was no evidence of bone formation in lased site. Nonlased site shows border of compact bone with underlying marrow tissue, marrow is composed of adipose and lymphomatous tissue. Bone is compact in nature with haversian canals, osteocyte lacunae and resting lines.

Discussion

Low level laser therapy (LLLT) is the application of light, usually a low power laser in the range of 1mW – 500mW to pathology to promote tissue regeneration, reduce inflammation and relieve pain. It is not similar to medical laser procedures. LLLT is not an ablative or thermal mechanism, but rather a photochemical effect comparable to photosynthesis in plants whereby the light is absorbed and exerts a chemical change.[19]

A biphasic response has been demonstrated many

times in LLLT research and the "Arndt-Schulz Law" is frequently quoted as a suitable model to describe dose dependent effects of LLLT.[20,21,22,23,24,25,26] Arndt-Schulz law states that weak stimuli slightly accelerate vital activity, stronger stimuli raise it further, but a peak is reached and even stronger stimuli suppress it, until a negative response is finally achieved. The increasing "stimulus" in case of LLLT is irradiation time or increased irradiance. A "biphasic" curve suggests that if insufficient energy is applied there will be no response (because the minimum threshold has not been met), if more energy is applied the then a threshold is crossed and biostimulation is achieved but when too much energy is applied then the stimulation disappears and is replaced by bioinhibition instead.[27]

The aim of our study was to investigate the effect of Gallium-aluminum-arsenide (GaAlAs) low level diode laser under single irradiation on healing of bone. Our study showed that irradiation with diode laser for 270 sec inhibited the resolution of inflammation and predominanatly filled the up the marrow spaces with the granulomatous tissue.

Saito S,[28] investigated the effects of low-power laser irradiation on bone regeneration during expansion of a midpalatal suture in rats. GaAlAs diode laser 100mW irradiation was applied to the midpalatal suture during expansion carried out over 7 days (3 and 10 minutes per day), 3 days (7 minutes per day) and 1 day (21 uninterrupted minutes on day 0). The bone regeneration in the midpalatal suture estimated by histomorphometric method in the 7-day irradiation group showed significant acceleration at 1.2- to 1.4-fold compared with that in the nonirradiated rats, and this increased rate was irradiation dose-dependent. One-time irradiation did not have any effect on bone regeneration. Authors conclude that low-power laser irradiation can accelerate bone regeneration in a midpalatal suture during rapid palatal expansion and that this effect is dependent on the timing and frequency of irradiation and not only on the total laser irradiation dosage. Soleimani M. et al[29] examined the influence of LLLT at different energy densities on BMSCs (bone marrow stromal cells) differentiation into neuron and osteoblast. Human BMSCs were cultured and induced to differentiate to either neuron or osteoblast in the absence or presence of LLLT. GaAlAs 810nm diode laser irradiation was applied at days 1, 3, and 5 of differentiation process at energy densities of 3 or 6 J/cm(2) for BMSCs. LLLT promoted BMSCs proliferation significantly at all energy densities except for 6 J/cm(2) in comparison to control groups on the seventh day of differentiation. Authors concluded that the effect of LLLT on differentiation and proliferation of BMSCs is dose-dependent. Sattayut S30 investigated the effect of 820 nm GaAlAs at energy densities of 4 J/cm2 and 19 J/cm2 on prostaglandin E2 (PGE2) production by myoblast cultures undergoing stimulation with interleukin I alpha (IL1). Authors concluded that 820 nm laser irradiation at 19 J/cm2 was found to inhibit that mechanism, while the lower energy density (4 J/cm2) failed to inhibit PGE2 production. Gross AJ[31] investigated the in vitro action of helium-neon (He-Ne) laser light on the cell cycle and the growth of rat kidney epithelial cell cultures. Study showed

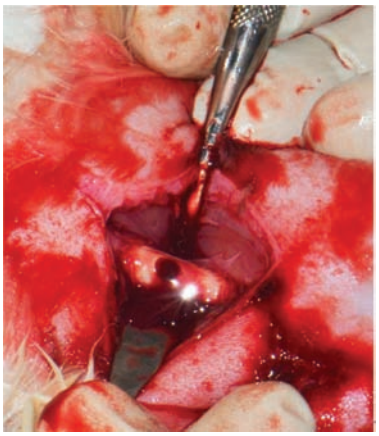


Fig 1: Femur

Fig 2: Diode laser

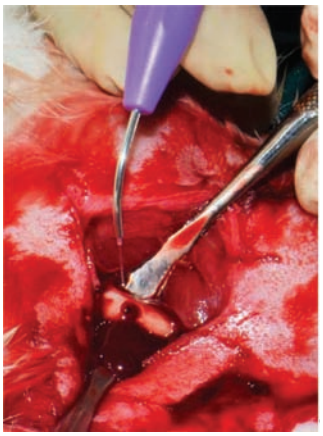


Fig 3: Application of Laser

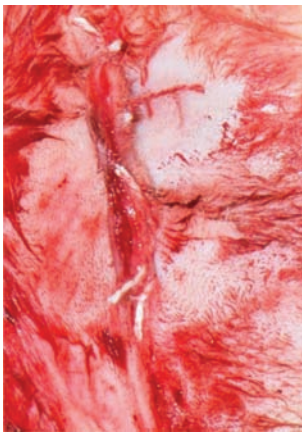
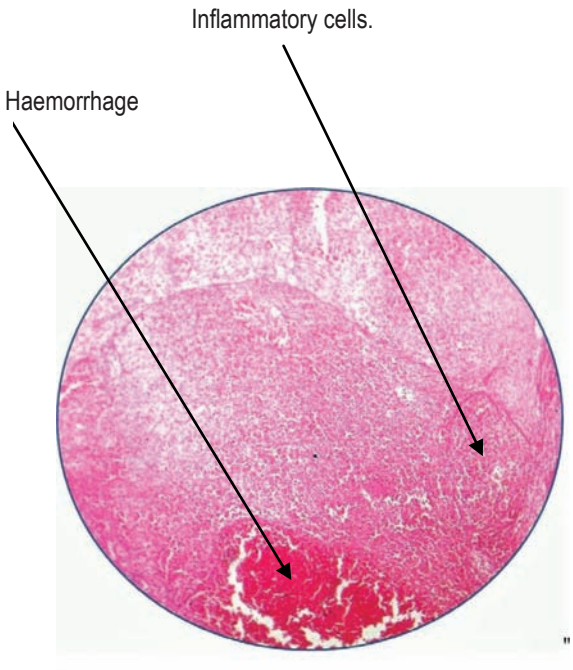


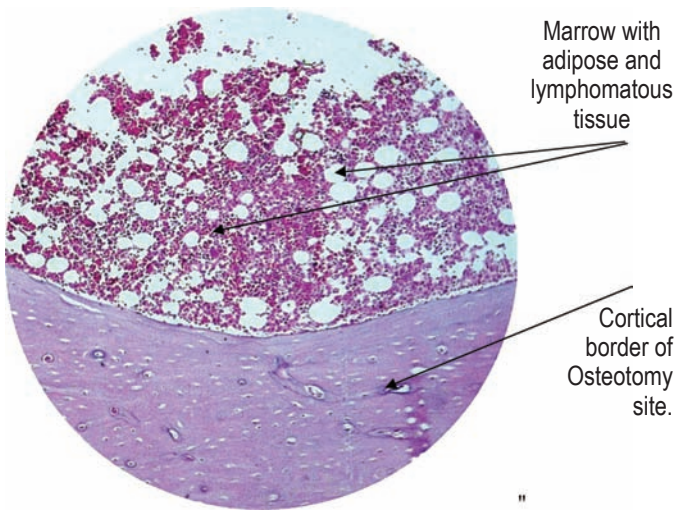
Fig 4: Suturing done

that repeated He-Ne irradiation (dose rate 40 mW/cm2) once a day in a dose range between 11.9 and 142 J/cm2 significantly inhibited cell growth, while daily irradiation with 4.7 J/cm2 had no effect. Authors concluded that the results support previous observations suggesting that laser light of low thermal energy interferes with cell cycling and may inhibit cell growth when irradiation is performed at doses of 11.9 J/cm2 or more.

Our study results are in agreement with Gross AJ[31] and Sattayut S[30], who showed that repeated He-Ne irradiation (dose rate 40 mW/cm2) once a day in a dose range between 11.9 and 142 J/cm2 significantly inhibited rat kidney epithelial cell growth & 820 nm laser irradiation at 19 J/cm2 was found to inhibit prostaglandin E2 (PGE2) production by myoblast cultures undergoing stimulation with interleukin I alpha (IL-1) respectively. The above studies were not related to bone & there were conducted on healing of soft tissues. Our study is also in agreement with study by Saito[28] who showed single irradiation group did not have any effect on bone formation compared to 7-day irradiation group (3 minutes and 10 minutes) which showed significant acceleration of bone regeneration. Total of 21 minutes were used in the single irradiation group. Our results are also in agreement with Soleimani,[29] using LLLT at 810 nm wavelength enhanced BMSCs differentiation into neuron and osteoblast in the range of 2–6 J/cm2 and at the same time increases BMSCs (Bone marrow stem cells) proliferation except for 6 J/cm2 pointing to the fact that failure of high dosage application. In above studies laser parameters used were different from our study parameters.

The assumption that is frequently made is that if a small dose of red or near infrared light produces a significant therapeutic effect, then a larger dose should produce an even more beneficial effect. This natural assumption is frequently not the case. Three possible explanations for the existence of the biphasic dose response were proposed. First hypothesis dealt with light mediated generation of reactive oxygen species which has been observed in many in vitro studies and has been proposed to account for the cellular changes observed after LLLT via activation of redox sensitive transcription factors.[32] It is well-accepted that ROS can have both beneficial and harmful effects[33]. Hydrogen peroxide at times used to kill cells in vitro.[34] Other ROS such as singlet oxygen[35] and hydroxyl radicals[36] are thought to be harmful even at low concentrations. The phenomenon of biphasic dose response in fact is well established in the field of oxidative stress.[37] If the generation of ROS can be shown to be dose dependent on the delivered energy fluence this may provide an explanation for the stimulation and inhibition observed with low and highlight fluences.





Another hypothesis that is put forward to explain the cellular effects of LLLT relates to the photolysis of nitrosylated proteins that releases free NO. Again the literature has many papers that discuss the so-called two-faced or "Janus" molecule NO[38,39]. Nitrous Oxide can be either protective or harmful depending on the dose and particularly on the cell or tissue type where it is generated.[40] The third hypothesis put forward was that the protective and stimulatory effects of light occur at low doses, but there is an additional pathway that leads to damaging effects of light that only occurs at high doses and undermines the beneficial effects of low doses of light. Low doses of LLLT were found to phosphorylate hepatocyte growth factor receptor (c-Met), and initiate signalling via cyclic AMP and Jun kinase and Src.[41] On the other hand, high dose LLLT was found to induce apoptosis via a mitochondrial caspase-3 pathway and cytochrome c release was attributed to opening of the mitochondrial permeability transition pore caused by high-level intracellular reactive oxygen species (ROS) generation.[42]

Conclusion

The results of the present study using 810nm, 90mW, for 270 sec for single session inhibited the healing of bone in two weeks compared to non-laser irradiated group. So the above parameters can be regarded as inhibitory dose for formation of bone.

Further scope of the study is to evaluate the effect of low level laser therapy using different energy densities for shorter duration of time in continuous wave.

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JSPIK

Pink esthetics in your smile design: by quantitative measurement of gingival elements- zenith points and its position in an esthetic smile line of maxillary dentition: a clinical study

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ABSTRACT

Aims: The purpose of this study was to measure and to compare the two clinical parameters - maxillary gingival zenith positions and gingival zenith levels of the lateral incisors and premolars in an apico-coronal direction between right and left maxillary anterior teeth for enhancing pink esthetics.

Methods and Material: A total of 53 samples were included in the study. The distance between the gingival zenith and gingival line were measured by using vernier caliper from the maxillary cast mounted on the semi adjustable articulator with orbital reference plane.

Results: The gingival zenith point of central incisor located 1.2 mm distal to Vertical Bisected Midline (VBM), lateral incisor located 0.6 mm distal to VBM i.e. near center, canine located 0.5 mm distal to VBM and premolar located 0.4 mm distal to VBM i.e. center. The gingival zenith level of the lateral incisor and premolar in relation to the gingival line drawn from tangent to the gingival zenith points were 1.2 mm, whereas Ist Premolar located 1.25 mm and II nd premolar located 1.6 mm. There were significant differences were evident between right and left gingival zenith points and levels.

Conclusions: The gingival zenith point and gingival zenith level from this study can be clinically used to establish pink esthetic smile in maxillary anterior dentition during periodontal surgery, crown lengthening or root coverage procedures, in prosthetic or in orthodontic treatment.

Key-words: Gingival zenith, Gingival line, Vertical Bisected Midline, apico coronal dimension, mesiodistal dimension.

Introduction:

To preserve, create or enhance a pleasing smile without impairing function is the utmost duty of a treating dentist. In order to achieve an ideal smile, we must need to evaluate the facial profile, lips, gingiva, teeth and their synergistic appearance altogether. These factors depend on absolute symmetry of facial and dental structures. In order to predict facial esthetic results, in relation to gingival contour related to smile, we should include evaluation of gingival contour in pre operative assessment. Gingival zenith, is the most apical aspect of the gingival margin, is considered as one of the important factor in defining gingival morphology.

Materials and methods:

A total of 53 patients who reported to the department of periodontics CSICDSR, were included in our study. The study was approved by IEC (CSICDSR/IEC/0043/2017). All participants were

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explained about the procedure and informed consent was obtained. The clinical parameters assessed were (i) maxillary Gingival Zenith Points (GZP) and (ii) Gingival Zenith Level (GZL) of lateral incisors and premolar in apicocoronal direction.

Inclusion criteria:

- Both genders
- Age limit 20-35,
- Esthetic smile line.

Exclusion criteria:

- Periodontal disease, gingival recession, or gingival hypertrophy, no loss of interdental papilla, spacing, crowding, existing restorations, and incisal attrition.
- Subjects with dental deformity, undergone any periodontal, surgical, or orthodontic treatment involving especially the maxillary anterior teeth.

Materials required:

- Alginate impression material
- Dental stone
- Face bow
- Semi-adjustable articulator (Whip mix 2200 series)
- Indelible marking pencil
- Digital vernier caliper

Based on the above criteria, patients with ideal smile were selected. Impression was taken with alginate and cast was poured with dental stone. Face bow transfer was done for every patient and the maxillary casts were mounted onto the semi-adjustable articulator (Whip mix 2200 series) relating it to orbital plane axis, refer figure 1. It is done to avoid any discrepancy affecting the relation of zenith points of maxillary teeth.

Gingival zenith points were made with indelible marking pencil for all the maxillary anterior teeth including premolar. Maxillary midline was drawn and marked as vertical bisected midline (VBM). Gingival line was drawn by connecting all the gingival zenith positions to the midline (refer fig 2). The lateral incisors and premolar relationship to the gingival line were evaluated by using Digital vernier caliper and readings were noted in mm.

Statistical analysis:

All measurements were made by single investigator. Each measurement was measured two times and the mean value was calculated. The statistical analyses were conducted with the level of significance (α) = 0.05. Independent t test was done to compare the gingival zenith position and gingival zenith level of maxillary anterior teeth between right and left.

Results:

The gingival zenith point of central incisor were located 1.2 mm distal to VBM, lateral incisor premo-

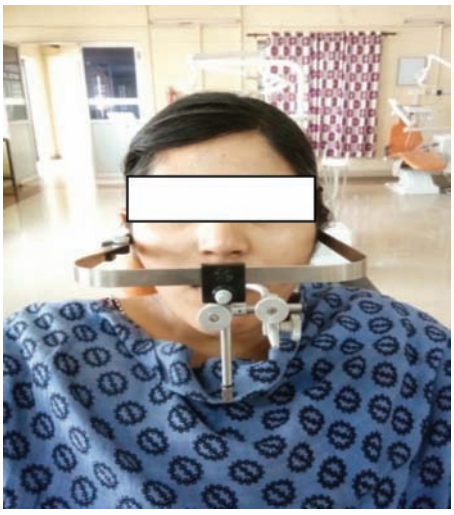

Fig 1: Facebow Transfer

Fig 2 Markings of gingival zenith point and its level

1 –gingival zenith point;

2-vertical bisected midline;

3-gingival level (drawn tangent line by connecting all the zenith points)

lar located 0.4 mm distal to VBM i.e. center (refer table 1).

The gingival zenith level of the lateral incisor and premolar in relation to the gingival line drawn from tangent to the gingival zenith points were 1.2 mm, whereas I st Premolar located 1.25mm and II nd premolar located 1.6 mm (refer table 3).

On comparing the right and left gingival zenith position and its level in the maxillary teeth, there were significant differences found between them (refer table 2, 4).

From this study, we can spot that right side of the maxillary anterior teeth had gingival zenith points and levels located few mm distal to the midline and apical when compared to the left side.

Discussion:

Smile is the perfect key to unlock everyone heart. Such a smile holds synergistic beauty of facial profile, lips, teeth and gingiva. Framing the teeth within the confines of the gingival architecture has a tremendous impact on the aesthetics of the smile[1]. Considering gingival esthetics in the maxillary dentition, it defines the zenith point and its level. Chu et al reported that GZP and the GZL of the lateral incisor and premolar relative to central incisors and canine can significantly influence the esthetic smile of the patients[2]. These details can significantly influence the esthetic appearance of a smile. The appropriate placement of the gingival zenith is critical, because it helps to determine the desired axial inclination of the tooth[3]. Subsequently, knowing the GZP of each maxillary anterior tooth from the VBM as well as the GZL of the lateral incisors can help facilitate a reference point during esthetic periodontal plastic surgery procedures.

In the present study we evaluated GZP and GZL of lateral incisor and premolar. Our study shows the mean GZP of 1.4 mm for central incisor, 0.5 mm for lateral incisor 1.0 mm for canine, 0.25 mm for Ist premolar and 0.25 mm for II nd premolar, which is consistent with the reports of Chu et al who evaluated the position of gingival zenith relative to the midline in maxillary anterior teeth in attractive smiles in 240 sites in 20 subjects. Duran et al who evaluated

Table 1: The results of gingival zenith position of maxillary anterior teeth in total population (n=53)

Tooth	Right side Mean±SD	Left side Mean±SD
Central Incisor	1.03±0.07	1.22±0.28
Lateral Incisor	0.61 ±0.21	0.58 ±0.20
Canine	0.51 ±0.11	0.52 ±0.16
Ist premolar	0.41 ±0.09	0.51 ±0.11
II nd premolar	0.31 ±0.11	0.43 ±0.09

Table 2: Results of gingival zenith position between right and left in total population:

Tooth	Right side Mean±SD	Left side Mean±SD	P-value
Central Incisor	1.03±0.07	1.22±0.28	0.00*
Lateral Incisor	0.61 ±0.21	0.58 ±0.20	0.60
Canine	0.51 ±0.11	0.52 ±0.16	0.05*
Ist premolar	0.41 ±0.09	0.51 ±0.11	0.00*
II nd premolar	0.31 ±0.11	0.43 ±0.09	0.20*

*significant difference at the 0.05 level (two tailed)

Table 3: The results of gingival zenith level of maxillary anterior teeth in total population (n=53)

Tooth	Right side Mean±SD	Left side Mean±SD
Lateral Incisor	1.11±0.27	1.21±0.21
Ist premolar	1.24 ±0.26	1.25 ±0.27
II nd premolar	1.61 ±0.37	1.58 ±0.39

Table 4: Results of gingival zenith level between right and left in total population

Tooth	Right side Mean±SD	Left side Mean±SD	P-value
Lateral Incisor	1.11±0.27	1.21±0.21	0.04*
Ist premolar	1.24 ±0.26	1.25 ±0.27	0.81
II nd premolar	1.61 ±0.37	1.58 ±0.39	0.68

*significant difference at the 0.05 level (two tailed).

zenith positions of premolar for 63 patients also had concurrent results[4].

In our study, GZP of central incisors and canine located distal to the VBM. Whereas for lateral incisors and premolar located central to the VBM, which is similar to the results obtained by Magne et al and zagar et al[5, 6]. In this present study, the mean apico-coronal position of the lateral incisor of GZL relative to adjacent GZP was 1.2 mm, which is more than results obtained by Charruel et al (0.68mm)[7].

When comparing the right and left side, the results from our study showed significant difference for the gingival zenith level of central incisor, Ist premolar and II nd premolar. The right side showed zenith point placed little apically, when compared to left side, which is in accordance to a study done by Pawar et al[1] who showed that the asymmetrical gingival contour is present in right and left side. This asymmetry may be more attractive than symmetrical beauty, as our human brain is less attractive to symmetry, making the faces appear passive and inert. This asymmetry may be due to occurrence of natural phenomenon[8].

In our study the central incisor and canine were found to be displaced little distally to the VBM which is in correlation to the reports of Rufenacht[9] and Goodlin et al[13].

Ideally in a class I occlusion, gingival zenith line of central incisor and canine should be at the same level, whereas the lateral incisor should be positioned slightly coronal. In a class II occlusion, the lateral incisor should be positioned more apically[9]. The results of our study also correlate with the studies done by Rufenacht[9] which shows that these tooth and root positions of the lateral incisors within the dental arch might affect the gingival contours. There were no significant differences between genders, which is similar to the study done by Humagain et al[10].

The findings of this current study shall help the clinician in preplanning the most complex situations which may demands high aesthetic accuracy in placing gingival contours during prosthetic or implant restorations, cosmetic periodontal surgery such as crown lengthening procedures and gingivoplasty for esthetic reconstruction and also help in the construction of surgical templates.

Gingival Zenith position can also act as reference point, in conjunction with other subjective and objective aesthetic parameters to aid in diagnosis, treatment planning, and in reconstructing a natural smile[11]. If a larger sample had been taken, the standardization of the clinical parameters would have been better.

Conclusion:

Knowing the GZP of each maxillary anterior tooth and GZL can help to facilitate a reference point during esthetic periodontal plastic surgery procedures, restorative procedures and orthodontic treatment. They add a blooming effect to the smiles created. It is our responsibility to understand the ramifications of these details and how they draw an impact on the smiles we create. Thus pink esthetics provides synergistic beauty to the smile.

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