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Waveone and one Shape Files: Survival in Severely Curved Artificial Canals

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Abstract- Nickel-titanium rotary instruments are preferred for their excellent flexibility, superelasticity and improved cutting efficiency but they can separate unexpectedly, especially in curved canals. Instrumentation with WaveOne and One Shape files was performed on 200 artificial canals divided in four equal groups. Glide path was created with PathFiles and G-files. Average lifespan and survival rate of the shaping files were tested, before and after a glide path creation. Average lifespan of WaveOne and One Shape files without a creation of a glide path was 10.25±2.50 canals and 4.1 (±1.37) canals, respectively and after the creation of a glide path – 17.50±2.12 canals and 4.6 (±1.30) canals. Average lifespan and cumulative survival of the tested files revealed significant difference. WaveOne files showed significantly higher resistance to fracture compared with One Shape files. Lifespan and survival rate of tested files increased after the creation of a glide path. Reciprocal motion increases significantly instruments life.

Keywords: *nickel-titanium instruments, waveone, one shape, pathfiles, g-files, glide path, lifespan, cumm-ulative survival, continuous rotation, reciprocating motion.*

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Emilia Karova [α] & Snezhanka Topalova-Pirinska [σ]

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Keywords: nickel-titanium instruments, waveone, one shape, pathfiles, g-files, glide path, lifespan, cummulative survival, continuous rotation, reciprocating motion.

I. Introduction

Mechanical instrumentation and thorough debridement of root canal space are essential prerequisites for successful endodontic treatment (Schilder, 1974; Hulsmann *et al*., 2005). Stainless steel files can be used for achieving this goal but recently nickel-titanium (NiTi) rotary instruments are preferred for their excellent flexibility, superelasticity and improved cutting efficiency (Chen *et al*., 2002; Sonntag *et al.,* 2003; Peters, 2004; Schäfer *et al.,* 2004). These instruments can minimize ledging and transportation, create more centered and rounded canal preparation and for these reasons are frequently chosen for instrumentation of curved root canals (Short et al., 1997; Bonaccorso *et al*., 2009; Cheung et al., 2009). Despite their undeniably favorable qualities, NiTi rotary instruments can separate unexpectedly, especially when they are forced into the canal or are overused (Sattapan et al., 2000; Arens *et al*., 2003). Fractures of NiTi rotary instruments are result of torsional stress or cyclic flexural fatigue (Di Fiore, 2007; Wu *et al*., 2011). Root canal anatomy, instrument design, manufacturing process, preparation technique and operator skill can affect the fracture mode and the fracture rate of these instruments (Parahos *et al.,* 2004; Parashos *et al.,* 2006; Shen *et al.,* 2009; Kim *et al.,* 2010; Zhang *et al*., 2010). The chance of removing the broken file is very low in some cases and sometimes may be impossible without compromising the endodontic treatment outcome (Hulsmann *et al*., 1999).

To reduce the separation incidence, manufacturers have developed new techniques to improve the physical and mechanical properties of their instruments. One important modification of the NiTi alloy, which makes rotary systems more flexible and more resistant to cyclic fatigue, and improves their cutting efficiency, is the M-Wire alloy (Dentsply Tulsa Dental, Tulsa, OK) (Gambarini *et al*., 2008; Alapati et al., 2009; Lopes *et al*., 2013). M-Wire is a NiTi alloy prepared by a special thermal process and is used in the production of WaveOne reciprocating files (Dentsply, Tulsa Dental Specialties, Tulsa, OK).

Reciprocating mode of rotation has been introduced recently with the intent to extend the lifespan of a NiTi instrument and its resistance to fatigue in comparison with continuous rotation (De-Deus et al., 2010; Varela Patino *et al*., 2010; You et al., 2010; Franco *et al*., 2011; Pedulla *et al*., 2013). WaveOne files are used with specific motor with unchangeable settings. The engine generates different angles of rotation - 170° counterclockwise and then 50° clockwise rotation with a speed of 350 rpm – which affect the fatigue resistance (Kim *et al*., 2012). The system consists of 3 sterile single-use files with noncutting modified guiding tips: small (ISO 21 tip and 6% taper) for small canals, primary (ISO 25 tip and 8% taper) for the majority of canals, and large (ISO 40 tip and 8% taper) for large canals. The last two have fixed tapers of 8% from D1 to D3, whereas from D4 to D16, they have a unique progressively decreasing percentage tapered design. Thus, flexibility is improved and the remaining dentin in the coronal two thirds of the canal is preserved. Another unique design features of the WaveOne files are the reverse helix and the two distinct cross-sections along the length of their active portions (a modified convex triangular cross section from D1 to D8 and a convex triangular cross section from D9 to D16). The design of the two WaveOne cross sections is further enhanced by a

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changing pitch and helical angle along their active portions (Webber *et al.,* 2011).

One Shape rotary NiTi files (MicroMega) work with continuous rotation and the producers try to increase their flexibility and to reduce instrument screwing effects by a variable cross-section along the blade of the instrument. They have 3 different cross-section zones: apical (with a variable 3-cutting-edge design), transitional (progressively changing from 3 to 2 cutting edges) and coronal (with 2 cutting edges). Anti Breakage Control (ABC) increases safety and avoids separation by unwinding of the instrument. The system consists of one sterile single file for root canal shaping (ISO 25 tip and 6% taper) with variable pitch and non-working (safety) tip.

File distortion and breakage, especially in severely curved canals, can be reduced or avoided not only by improved design and mechanical properties of the instruments, but by manual preflaring of the root canal and creation of a glide path, as well. There is a strong evidence in the literature (Roland *et al.*, 2002; Berruti *et al.*, 2004; Varela-Patino et al., 2005; Zarrabi *et al.,* 2010) that fracture incidence might be reduced as a result of the preliminary enlargement of the root canal diameter. Thus, the size of the canal becomes bigger than or at least the same size as the tip of the first shaping rotary instrument used (Berruti et al., 2004; Varela-Patino *et al.*, 2005). Hand-operated and engine-driven instruments can be used for glide path creation. Recently, two new rotary NiTi systems have been introduced for this purpose: PathFile System (Dentsply Maillefer) and G-Files (MicroMega).

The aim of the present study was to compare the survival of WaveOne and One Shape files used for the instrumentation of severely curved artificial canals, before and after the creation of a glide path.

II. Materials and Methods

Canal preparation was performed on 200 Endo-Training Block simulators (Dentsply Maillefer) with an apical diameter of 0.15 and a 0.02 taper, a 65 degree curvature and a 7.5 mm curvature radius.

The canals were divided in four equal groups: 1st group – shaped with WaveOne (Dentsply Maillefer) reciprocating files; 2nd group – shaped with One Shape (Micro Mega) files; 3rd group – preflared with PathFile System (Dentsply Maillefer) and shaped with WaveOne files; 4th group – preflared with G-Files (Micro Mega) and shaped with One Shape files.

Average lifespan and cumulative survival at the time of WaveOne and One Shape files were tested, before and after a glide path creation.

Following the instructions of the producer all files were operated using The WaveOne™ Endodontic system (Dentsply Maillefer), which is pre-programmed with settings for the WaveOne reciprocating file system. The One Shape files were used with a rotation speed of 400 rpm and torque 2.0gr/cm$^{2.}$ PathFiles and G-files worked at one and the same rotation speed (300 rpm) and torque (0.6gr/cm^2). The amount of pressure applied to all files was the pressure that could be applied to a sharp #2 pencil without breaking the lead. The files were never forced into the canal.

Initially, all canals were scouted to full working length with a #10 hand K-file. In the first group all canals were shaped with the small (ISO 21 tip and 6% taper) WaveOne file, following the instructions of the producer (a #10 hand K-file to be very resistant to movement at the initial inspection of the root canals).

In the second group shaping was performed with One Shape files (ISO 25 tip and 6% taper).

In the third group a glide path was created at the beginning with PathFile System. It consists of three instruments with a square cross section, four cutting angles, 21-25-31 mm length, a 0.02 taper and a size of the tip ISO 13, 16 and 19. Later, the canals were instrumented with the small (ISO 21 tip and 6% taper) WaveOne file.

In the fourth group G-Files were used for glide path creation. G-Files consist of two instruments 21-25-29 mm long, with a 0.03 taper, a size of the tip ISO 12 and 17 and a variable cross-section throughout the length of the instrument. The 3 cutting edges are on three different radiuses relative to the axis of the canal, leaving a large and efficient area for upward debris removal. Shaping was finished with One Shape files (ISO 25 tip and 6% taper).

WaveOne and One Shape files worked till fracture occurred.

During mechanical instrumentation each file was coated with Glyde™ (Dentsply Maillefer) to act as a lubricant, and copious irrigation with 5.25% NaOCl was carried out.

The instrumentation of all canals was performed by a single operator.

III. Results

a) Average Lifespan of Shaping Files

i. *Comparison between WaveOne and One Shape files*

Fifteen files were used in canals' preparation and fourteen of them broke: 4 WaveOne files and 10 One Shape files. The longest lifespan of a single WaveOne file was 13 canals and of a single One Shape file - 7 canals. Respectively, the shortest lifespan was 7 and 2 canals.

The average lifespan of one WaveOne file was 10.25±2.50 canals and of a single One Shape file - 4.1±1.37 canals. The difference was statistically significant ($p<0.001$) (t-test).

ii. *Comparison between WaveOne and WaveOne+PathFiles*

Eight WaveOne reciprocating files were used in canals' preparation and six of them broke: 4 files before the creation of a glide path and 2 after the initial enlargement of the artificial canals with PathFile System. The longest lifespan of a single file from the first group was 13 canals and from the second group – 19 canals. The shortest lifespan was measured in the first group and was 7 canals.

The average lifespan of one WaveOne file without a creation of a glide path was 10.25±2.50 canals and after a creation of a glide path – 17.50±2.12 canals. The difference was statistically significant ($p<0.05$) (t-test).

iii. *Comparison between One Shape and One Shape+G-Files*

Nineteen files were used during the instrumentation and 18 of them broke: 10 in the group without a glide path and 8 – after the preliminary enlargement with G-Files. The longest lifespan in the first group is 7 canals and in the second – 6 canals. Two canals were the shortest lifespan in both groups.

The average lifespan of a single One Shape file without a creation of a glide path was 4.1 (±1.37) canals and after a creation of a glide path – 4.6 (±1.30) canals. The difference was statistically insignificant ($p>0.05$) (t-test).

iv. *Comparison between WaveOne+PathFiles and One Shape+G-Files*

Twelve shaping files were used after the initial enlargement of the canals – 3 WaveOne files and 9 One Shape files. During the instrumentation ten of them broke: 2 WaveOne files and 8 One Shape files. The longest lifespan of a single file from the first group was 19 canals and from the second group – 6 canals. The shortest lifespan was measured in the second group and was only 2 canals.

After the creation of a glide path, the average lifespan of one WaveOne file was 17.50±2.12 canals and of a single One Shape file–4.63±1.30 canals. The difference was statistically significant ($p<0.001$) (t-test). The results of the average lifespan of the tested shaping files can be summarized in Table 1.

Table 1 : Average lifespan of WaveOne and One Shape rotary files

№	Preparation technique	Number of broken files	Highest number of successfully treated canals	Lowest number of successfully treated canals	Average lifespan
1	WaveOne	4	13	7	10.25±2.50
2	WaveOne+PathFile	2	19	16	17.50±2.12
3	One Shape	10	7	2	4.1 (±1.37)
4	One Shape+G-Files	8	6	2	4.63±1.30

b) Cumulative Survival at the Time of Shaping Files

The survival rate for one WaveOne files was 75% at the instrumentation of the 8th canal, 50% at the 11th one and 25% at the shaping of the twelve canal.

The cumulative proportion surviving at the time for One Shape was 90% at the instrumentation of the 3rd canal, 70% at the 4th one, 30% at the 5th and 10% at the 6th canal. All files were broken at the preparation of the 8th canal.

The cumulative survival for WaveOne files, after the creation of a glide path, was 50% at the instrumentation of the 17th canal and all files were separated at the instrumentation of the 20th canal.

The survival rate for One Shape files, after the creation of a glide path, was 88.9% at the instrumentation of the 3rd canal, 66.7% at the instrumentation of the 5th canal, 26.7% at the preparation of the 6th canal and all files were separated at the instrumentation of the 7th canal.

i. *Comparison between WaveOne and One Shape files*

The comparison between the two systems reveals significant difference ($p<0.05$). At the 6th canal the cumulative survival of one WaveOne file was 100%, while it was only 10% for a single One Shape file. At the 8th canal 75% of WaveOne files were intact but all One Shape files were broken.

ii. *Comparison between WaveOne and WaveOne+PathFiles*

After the creation of a glide path, all WaveOne Files were intact (100% survival) at the instrumentation of the 14th canal, while all of them from the first group (without a glide path) were broken. The log-rank test presented a significantly longer survival ($p<0.05$) for the instruments used after a glide path was created.

iii. *Comparison between One Shape and One Shape+G-Files*

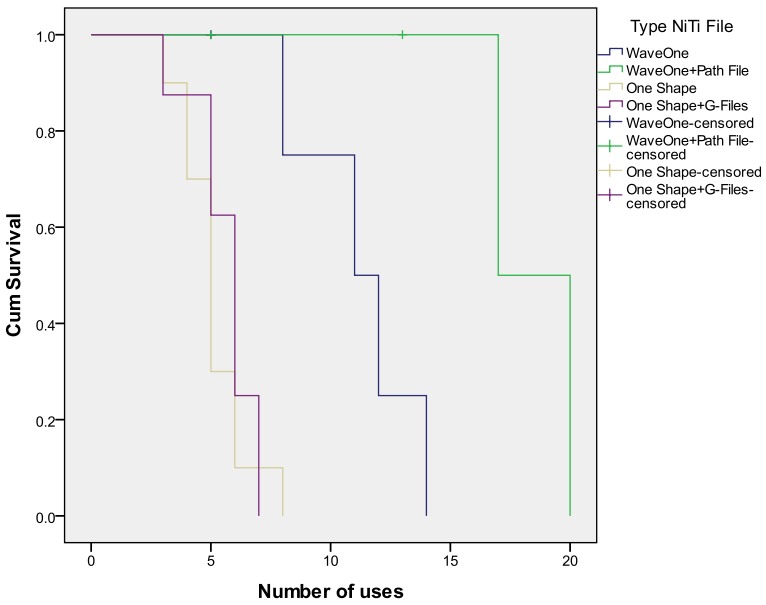
At the shaping of the 5th canal, after the creation of a glide path, the cumulative survival of a single One Shape file (66.7%) was twice as high as in the first group (30%).

Although the survival rate increased after the preliminary enlargement of the canal diameter, the difference between the two groups remained insignificant ($p>0.05$).

iv. *Comparison between WaveOne+PathFiles and One Shape+G-Files*

When comparing the results from both groups, it is found that at the instrumentation of the 7th canal all WaveOne files were intact (100% survival), while all One Shape files were broken. After the creation of a glide path, the log-rank test presented a significantly longer survival for the WaveOne instruments when compared with the One Shape files ($p<0.05$).

The survival curves of the tested instruments are presented on Fig. 1.



Figurer 1 : Survival curves of WaveOne and One Shape files

IV. Discussion

Our study was carried out on standardized artificial canals, which are expected to minimize the influence of other variables (Yao et al., 2006). We used Endo-Training Block simulators with high degree curvature in the apical 1/3 because we wanted to trace out and to compare the survival of WaveOne and One Shape rotary files under the conditions of greater stress. It is well documented in *in vitro* investigations that a stronger curvature and a smaller radius of the root canal increase the risk of rotary instrument fracture (Pruett et al., 1997; Zelada *et al.,* 2002; Martin *et al*., 2003). Despite the proven beneficial characteristics of the nickel-titanium rotary instruments, the risk of their separation, especially during the instrumentation of severely curved canals is higher (Iqbal *et al*., 2006; You et al., 2010; Setzer *et al.,* 2013). Fracture of NiTi files is a result of flexural and torsional failure. Flexural fracture is due to repeated compression and tension in curved canals that lead to cyclic flexural fatigue of the metal and the initiation of cracks from the outer surface of the instrument (Berutti *et al.,* 2006). In majority of cases, torsional fracture occurs in the last millimeters of the file when the tip or any other part of the instrument binds to the canal walls whereas the handpiece keeps turning (Sattapan *et al*., B 2000; Arens *et al*., 2003; Di Fiore, 2007; Varela Patino *et al.,* 2010). Consequently, NiTi files exposed to torsional stress are more susceptible to fracture and can break at a lower cyclic fatigue (Cheung *et al.,* 2005), and at the same time, torsional resistance decreases in used files (Parashos *et al*., 2006).

The observations in our study are similar to the findings in the works of Pruett et al., Varela Patino *et al*. and Tygesen *et al*. (Pruett *et al*., 1997; Tygesen *et al*., 2001; Varela Patino *et al*., 2005). Instrument breakage occurred in the apical portion of the canal, a few millimeters from the tip of the file, at the point of maximum flexure within the canal, where the stress is greatest.

The choice of the instruments compared in our investigation was not accidental. Our attention was directed to WaveOne and One Shape NiTi rotary files because they are sterile single-file systems and have non-working (safety) tip and variable cross-sections along the blade of the instrument. At the same time, they work with different mode of rotation – reciprocating (WaveOne) and continuous (One Shape). Both producers claim their products are safe and ensure an effective apical progression with low risk of fractures and obstructions. In fact, the incidence of their separation in clinical practice can increase as s result of overusage due to their high cost.

The examined files have different size of the tip because of two reasons: firstly, the One Shape files are offered only in one size and secondly, the tip size of the WaveOne file was chosen strictly following the instructions of the manufacturer - to use the small

WaveOne file, if #10 hand K-file doesn't easily move toward the terminus of the canal.

Sometimes, shaping of severely curved canals is a big challenge. That is the reason most clinical guidelines and manufacturers' recommendations for instrumentation with rotary NiTi instruments to claim reduction of canal curvature. It can be achieved either by creating straight-line access, which is not always possible, or by initial enlargement of the canal. The preliminary creation of a glide path can be regarded as a crucial step during chemomechanical endodontic procedures which reduces the interference of the shaping instrument with canal walls and makes the subsequent use of the larger rotary NiTi instruments safer and more effective (Roland *et al*., DD, 2002; Ruddle, 2005; Di Fiore, 2007; Peters e*t al.,* 2010). The probability of canal modifications and aberrations seems to be significantly reduced (Berruti *et al.,* 2004; Varela Patino *et al.,* 2005; Berruti *et al.,* 2012).

The role of the initial creation of glide path is clearly demonstrated in our investigation, as well. After the preliminary enlargement of the artificial canals the average lifespan and the cumulative survival of WaveOne and One Shape files were increased.

In the couple WaveOne-WaveOne+PathFiles the difference between the average lifespan in the two groups was statistically significant ($p<0.05$). From 10.25±2.50 canals it raised to 17.50±2.12 canals, the number of broken files in the second group decreased twice and the highest lifespan values were found here – 16 and 19 canals. The log-rank test presented a significantly longer survival ($p<0.05$) for the instruments used after a glide path was created. In this group all WaveOne files were intact at the instrumentation of the 14th canal while all the files from the first group were broken; 50% of the instruments remained safe at the shaping of the 17th canal and the files didn't break until the preparation of the 20th canal.

The couple One Shape-One Shape+G-Files reveals the same tendency. The average lifespan was increased from 4.1 (±1.37) to 4.6 (±1.30) canals after the creation of a glide path but the difference was insignificant ($p>0.05$). We found the greatest number of fractures in the study – 10 and 8 files, respectively and the shortest lifespan of only two uses. At the instrumentation of the 5th canal in the second group the cumulative survival of a single One Shape file (66.7%) was twice as high as in the first group (30%) but the difference between the two groups remained insignificant ($p>0.05$).

The great difference in the results obtained for WaveOne and One Shape files can be explained to some extent with the characteristics of the two NiTi rotary systems used for glide path creation. They are claimed by the producers to create a good combination of flexibility, strength and efficacy that allows a safe and fast use even in severely curved and/or calcified canals. The last files used from the two systems have different tip size (PathFile – ISO 19 and G-Files – ISO 17) and create different apical size of the canal. For the WaveOne group it is closer to the size (ISO 21) of the shaping file used when compared with the One Shape group (ISO 25). Consequently, under one and the same conditions, the stress accumulated on One Shape files is greater and the separation incidence increases.

The great number of uses of WaveOne files can be attributed not only to the creation of a glide path but to the specific reciprocal way of rotation and their design, as well. It is well documented that the reciprocating rotation decreases the impact of cyclic fatigue on nickel-titanium rotary instrument life and the incidence of instrument fractures (in resin blocks and natural teeth) is lower (Varela-Patino *et al*., 2008; Varela-Patino *et al*., 2010; You et al., 2010; Pedulla et al., 2103). The survival of an instrument is directly proportional to the stress accumulated during work in the root canal (Berutti *et al*., 2003).

In the couple WaveOne-One Shape a statistically significant difference between the average lifespan of the two groups was found out. For one WaveOne file it was 10.25±2.50 canals and for a single One Shape file – only 4.1±1.37 canals. The tendency is preserved when the comparison was made after the creation of a glide path. The average lifespan of one WaveOne file was 17.50±2.12 canals and of a single One Shape file –4.63±1.30 canals and again the difference was statistically significant ($p<0.001$).

The results from the cumulative survival are similar. The comparison between the two systems reveals a huge difference. In the couple WaveOne-One Shape 75% of WaveOne files were intact at the shaping of the 8th canal while all One Shape files were broken. When a glide path was created, all One Shape files were separated at the instrumentation of the 7th canal but 100% of WaveOne files survived.

The findings from our study undoubtedly demonstrate that reciprocating motion reduces torsional stress and avoids taper-lock due to the unsymmetrical repeating of the clockwise and counterclockwise rotations (Yared et al., 2001; You *et al.,* 2010). Our results are in agreement with the findings of the work of Varela Patino *et al.* (Varela Patino *et al*., 2010) in which the incidence of instrument fracture in blocks of resin was lower with alternating rotation than with continuous rotation. The mean number of uses in their study was 10 with alternating movement compared with 4-5 uses with continuous rotation. Torsional stress is additionally decreased when a glide path is initially created because the area on which the stress is exerted on is shifted from the tip of the file to its body. Reciprocating motion raises our expectations for longer survival of the files and gives us more comfort and safety, especially during shaping of severely curved root canals.

V. Conclusion

In conclusion, within the limitations of this study, WaveOne files showed significantly higher resistance to fracture compared with One Shape files. Instrumentation with files with reciprocal motion increases significantly instruments life and makes them safer during shaping of root canals. NiTi rotary instruments can be used for creation of a glide path as a result of which the lifespan and survival rate of WaveOne and One Shape files increase.

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To Evaluate the Effect of Probiotic Mouthrinse on Plaque and Gingivitis among 15-16 Year Old School Children of Mysore City, India- Randomized Controlled Trial

By Dr. Sandhya Purunaik, Dr. Thippeswamy H M & Dr. Shrinivas Shripathrao Chavan

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Objectives: This study aimed to investigate the efficacy of a Probiotic mouthrinse in reducing plaque and gingivitis among schoolchildren aged 15-16 years.

Methods: This was a randomized, controlled, double blind clinical trial. 90 subjects granting their parental informed consent and willing to participate completed the trial. The sample was randomized by computer generated table into Group A –0.2% of chlorhexidine mouthrinse, Group B – Probiotic mouthrinse, Group C – Placebo mouthrinse. Products were masked as regards color. Intervention protocol consisted in supervised rinsing of 20 mL/day for 60 seconds twice daily for 14 days. Plaque and gingival indexes were used to assess the efficacy variables, measured at baseline and after intervention by calibrated examiner. Data were statistically analysed.

GJMR-J Classification: *NLMC Code: WC 195, WN 230*

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Abstract- ***Introduction:*** Probiotic concept of using beneficial bacteria has recently gained popularity in medical research. New methods such as probiotics has given a new dimension for both general and oral health.

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Results: It was found that both Probiotic and chlorhexidine mouthrinses were able to significantly reduce plaque and gingival levels after 14 days ($p<0.05$).

Conclusion: probiotic mouthrinse showed significant reduction in plaque score and gingival level.

1. Gingivitis has been largely distributed among children and adults. Hence, it becomes important to consider alternatives for better oral health care.
2. A new possibility; to control plaque and gingivitis levels by means of a natural product that seems to overcome adverse effects of chlorhexidine mouthrinse such as altered taste and tooth staining is provided.
3. The product investigated was proven to be efficient and safe in a 14-day treatment. Also, it was well accepted by study participants.

I. Introduction

More than 1000 bacterial species have been identified from the human mouth. These microorganisms are easily grown and produce dental plaque in the mouth environment, due to the considered as a microbiota consisting on average of more than 400 species in each gram of plaque removed from the teeth. These live together within a biofilm community through the exploitation of very specific ecological niches[1.]

Dental disease such as dental caries and periodontal disease remains a "silent epidemic" in the world that threatens children and adults. The oral streptococci especially mutans streptococci are related with the development of caries. The adhesion of oral streptococci such as Streptococcus mutansto tooth surfaces has the major role in their pathogenicity. Going along with the increasing antibiotic resistance of bacteria, new methods such as whole bacteria replacement therapy for decreasing of oral cavity pathogens must be investigated.[2]

The mere spell of the word microorganism often gives a threat of health hazard. But, friendly microorganisms called Probiotics have changed this concept and have given a new dimension for both general and oral health[3]

The definition of "probiotics" has been adopted by the International Scientific Association and the World Health Organization: "Live microorganisms, if administered in adequate amounts, confer a health benefit on the host"[4]

The basic rationale behind the tautology of probiotics was that the human body lives in a heavily contaminated environment associated with millions of bacteria and probiotics can be utilised by replacing pathogenic microorganisms with healthy ones. This concept of using beneficial bacteria has gained much popularity in the field of medical research in recent years where antibiotic resistance is an increasing global problem[5]

The first species introduced into research were Lactobacillus acidophilus and Bifidobacteriumbifidum , and among a number of potential benefits that have been proposed are reduced susceptibility to infections , reductions to allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values.Within dentistry, previous studies with lactobacilli strains such as L.rhamnosus , L. acidophilus and L. casei, L.reuteri or a lactobacilli mix have revealed mixed results on oral microorganisms.[6]

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To our knowledge in India, none of these formulations are readily available for oral health, so there exists a need to explore easily available alternative approach to bacterial mediated oral disease such as gingivitis.

Hence this study was undertaken to test the hypothesis that Short term administration of probioticmouth rinse is effective in reducing plaque and gingivitis

II. Materials and Methods

a) Sample size calculation

This study is a double blind Randomized controlled trial and powered to evaluate the effect of probiotic mouth rinse on plaque and gingivitis.From a review of key papers the ideal sample size toassure adequate power for that Randomized Controlled Trial was calculated considering potential mean difference of 1.5 between control and test groups for the difference between subject values on Quigley Hein Plaque index. It was determined that 30 subjects pergroup would be necessary to provide80% power with an α of 0.05.

i. *Subject population, inclusion and exclusion criteria*

Subjects were selected from the populationby simple random sampling. In brief, the 90 eligible subjects were thoroughlyinformed of the nature, potentialrisks and benefits of their participationin the study and signed a termof Informed Consent.

b) The inclusion criteria were as follows

- School children between 15-16 years of age as per school records.
- School children willing to participate.
- School children with parental consent
- School children with no recent antibiotic therapy(within 4 weeks)

c) The exclusion criteria were as follows

- Children undergoing orthodontic treatment
- Children suffering from any systemic illness.
- Children using any other commercially available mouth rinse or probiotic products.
- Children using any other oral hygiene aids other than routine teeth brushing.
- Children with mixed dentition

A complete clinical oral assessment of all subjects was carried out based on inclusion and exclusion criteria for selection of study subjects.

ii. *Experimental design, allocation concealment*

The medical and dental records of all subjects were recorded by a questionnaire.

In this double blinded randomized placebo controlled clinical trial , subjects were enrolled and assigned to a computer generated table by the examiner who assigned the coded mouthrinses according to treatment groups after Baseline examination into :

Group A – 0.2% of CHX mouthrinse
Group B – Probiotic mouthrinse
Group C – Placebo mouthrinse

The subjects and the examiner were blinded regarding the product allocation.Knowledge of the randomization list obtained by computer generated table was limited to the study coordinator.

d) Preparation of Mouthrinses

JSS University pharmacy prepared the mouthrinses in undistinguishable packets and sent them to the study coordinator, who marked the code number of each subject on the packets, according to the therapy assigned and gave them to the examiner. The random allocation sequence was generated by the clinical investigator.To maintain full blinding of the results , the randomization table was held by the study coordinator remotely from all the assessment and was not broken until the data was collected and analysed. The randomization was concealed by using sequentially numbered ; identical appearing containers to subject assigned treatment. The mouthrinses were decoded after the data was analysed.

Probiotic product: probiotic mouthrinse was prepared by using commertially available probiotic product Darolac (Aristo pharmaceuticals, india) containing 1 gm powder of 1.25 billion freeze dried combination , it comprised of a mixture of , *Lactobacillus acidophilus, lactobacillus rhamnosus, bifidobacteriumlongum, and Saccharomyces boulardii* .Each sachet powder was dissolved in 20ml of water in a measuring cup and used as a mouth rinse[5].The placebo mouthrinse was prepared using distilled water.

*Chlorhexidine*gluconate mouthwash (Proprietary name: Clohex, concentration 0.2%) was procured from the market and given to the pharmacy manufacturing center. It was then diluted and the final concentration of *Chlorhexidine*gluconate was 0.2% such that 20 ml was dispensed at one time. Both solutions were made of identical colors to eliminate bias

Investigators calibration: The examiner participated in calibration exercise that was performed by taking measurement in duplicate at randomly chosen teeth in subjects who were not included in the study. Calibration was accepted when the results were identical on >85% of occasions

Treatment protocol : when the subjects volunteered for the study and before they received a packet containing mouthrinses and instruction for use, baselineplaque index Tureskey modification of Quigley & Hein Plaque index(QHI)[7] and gingival index [8](Loe H. and Silness P., 1963) were recorded by clinical examination. Gingivitis of the buccal and lingual marginal gingiva and

interdentalpapillae of all scorable teeth wasscored using the Loe–Silness Gingival Index(Loe&Silness 1963) in which thegingivae are scored on a four-pointscale from 0 (absence of inflammation) to 3 (severe- inflammation). Supragingival plaque was scored onthe buccal and lingual surfaces of allscorable teeth using the Tureskymodification of the Quigley–HeinPlaqueIndex (Turesky et al. 1970).Following disclosing with an erythrosine solution, plaque was scored on a six-point scale from 0 (no plaque) to 5 (plaque covers two thirds or more of the tooth surface).

Each subject was given one of the test products with a given code according to the assigned group. 20 ml of mouth rinse was dispensed for each individual using a measuring cup & subjects were instructed to swish the mouth rinse for 60 seconds & then expectorate. The procedure was performed once daily morning, after breakfast & was supervised by the examiner by visiting the school every day in the morning. The subjects were given with the assigned treatment group the mouthrinses in a packet and instructed to repeat the procedure before retiring to bed at night for the next 14 days. During the intervention period,no influence on personal oral hygiene procedures was exerted the subjects were encouraged to maintain routine oral hygiene & also instructed to maintain strict compliance.

A day after the 14 days of intervention, gingival & plaque indices were recorded using same indices by same examiner .This study protocol was approved by the JSS University Research Ethics Committee.

Clinical monitoring: clinical monitoring was performed by single examiner at baseline and 14 day.

Monitoring of compliance and adverseevents: The monitoring of compliance was assessed by instructing the subjects to return the old packets containing mouthrinses and received a new packets of mouthrinses. The single examiner was responsible for conducting the enquiry on adverse events and also monitoring of compliance During the study period no dropouts and withdrawals were encountered.

Primary outcome variables: All clinical measurements were obtained in all subjects at baseline and 14 day. It was defined that the primary outcome variable to determine the superiority of one treatment over the other would be differences between groups in the reduction of plaque and gingival index compared from baseline to follow up.

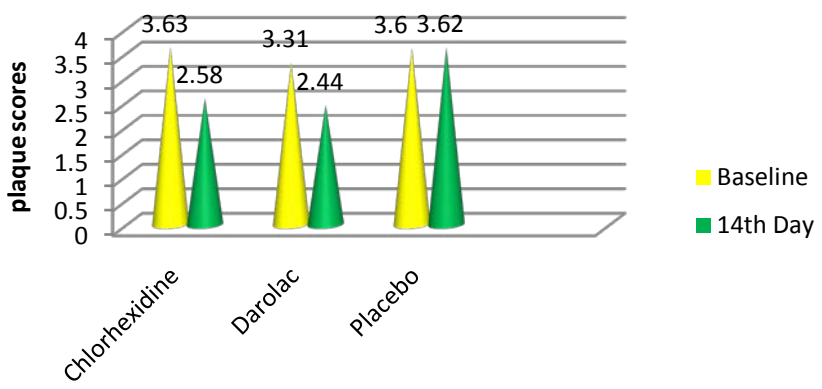
Statistical analysis: The significance of difference within each group (over the course of study) was sought using paired student t test . Data was analysed with statistical SPSS software package.The level of significance was set at 5%.

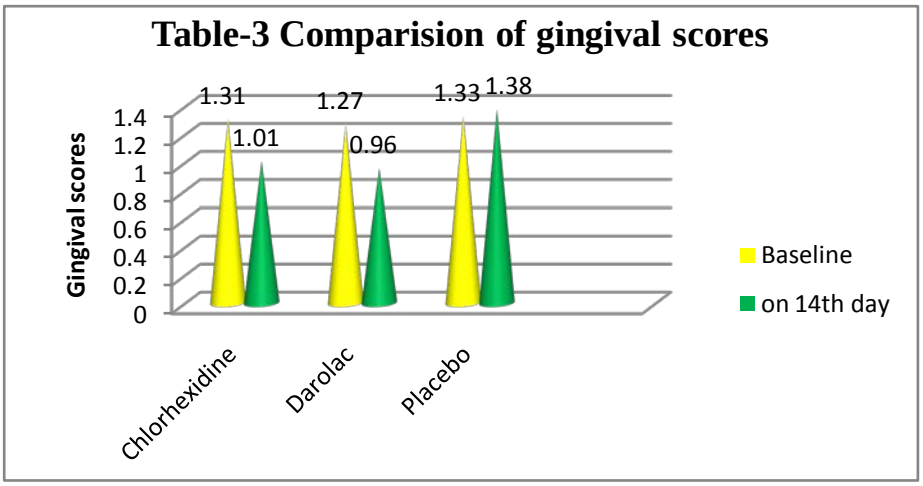
III. Results

90 subjects were included in entire study with 30 subjects allocated in each group . On comparison of plaque scores from Baseline to 14th day there was astatisticallysignificant reduction with mean differences of 1.05 and 0.87 for chlorhexidine and probiotic group($p<0.05$).The reduction in mean plaque score was found to be greater for chlorhexidine group than the probiotic group.But their was no statistically significant reduction in placebo group for plaque scores.

On comparison of gingival scores from baseline to 14th day there was a statistically significant reduction with mean differences of 0.30and 0.31 for chlorhexidine and probiotic($p<0.05$). Although the probiotic-mouthrinse was significantly more effective than chlorhexidine at 14 day ($p<0.01$). But their was no statistically significant difference in placebo group.

Table-2 Comparision of plaque scores





IV. Discussion

This controlled comparative clinical trial demonstrated that the probioticmouthrinse and the chlorhexidinemouthrinse produced significant reductions in supragingival plaque and gingivitis when used as adjuncts tosubjects' usual mechanical oral hygiene procedures. These findings add to the body of data supporting the effectivenessof these two antiplaque/ antigingivitis products.The finding that the respective 14 day plaque and gingivitisreduction indicates that the two active mouthrinseshad omparable clinical effectiveness. The data in the study compares favourably with those from the study performed by Krasse et al[9], A 14-day intake of L. reuteriled to the establishment of the strain in the oral cavity and significant reduction of gingivitis and plaque in patients with moderate to severe gingivitis.

A gingival infection is caused by a mix of Gram positive and Gram negative species and characterized by pronounced leucocyte infiltration and inflammatory exudation in the marginal area. The mechanism of probiotic action in the oral cavity is not fully understood, but is commonly explained by the combination of Local and systemic immunomodulation as well as non immunologic defense mechanisms. The study reported by SvanteTwetman et al[10] , that have examined Short-term effect of chewing gums containing probiotic Lactobacillus reuteri on the levels of inflammatory mediators in gingival crevicularfluid.The authors reported significant reduction in Cytokines TNF-α and IL-1β , which are considered central mediators of pro-inflammatory cascade causing damage. This result, to some extent explained the mechanism of probiotic action in the oral cavity.

In the light of present findings, our study results also showed a significant reduction in gingival status on short – term administration of probiotic mouthrinse. The results are also in consistent with study doneby to Kanget al[11], studies on three strains ofL. Reuteridemonstrated a centrifuged supernatant inhibitory effect on periodontopathic and cariogenic bacteria, all three inhibited the growth ofthe periodontopathic bacteria and S. mutansby more than 90%. This novel observation was also revealed in a study done by Margarita et al[12], it was concluded that L. Reuteri containing probiotic tablets are able to colonize the saliva and the subgingival habitat of some gingivitis patients. The use of the probiotic was associated with a reduction of total bacterial counts in saliva and reductions in the numbers of selected periodontal pathogens.

It is probably the production ofsome compounds such as bacteriocin or biosurfactant, which is the most likely reason for the antimicrobial effect of the probiotic powder[13,14]. Another crucial realm of probiotic bacterial clinical impact is mechanism by which they act, thus improving the intestine and over all health .Several reports have documented the ability ofprobiotic bacteria to inhibit; cell association, colonization and invasion by pathogenic bacteria.[13, 15, 16]

In a study done by khanfari[17], the research aimed to investigate the induction or reduction of *S. mutans*growth as it is a dominant bacterium producing dental plaque. In conclusion, the results showed that probiotic strains and probiotic chocolate can inhibit the growth of oral isolates of *S. mutans,* but their capacity differed significantly between the various strains.

In our study we used probioticmouthrinse containing combination of lactobacillus strains and strain of bifidobacterium and Sacchromyces that contains 1.25 billion freeze dried bacterial combination.It is possible, in the complex environment of the human mouth , that probiotic "cocktails" of multiple strains would be more effective than any single probiotic agent. This combination of probiotic strain was similar to those used by Haukoja et al[16].The author reported the clinical treatment of periodontitis and gingivitis seems to be a potential target for probiotic lactic acid bacteria or bifidobacteria.A basic prerequisite to be an oral probiotics is the ability to bond and inhabitant over the oral mucosal surfaces. Action of the probiotic strains on the oral cavity is dubious as oral mucosa is not their

innate habitation. The study proved that lactobacilli strains maintain oro microbiological balance. But the there is negligible proof that these lactobacilli strains are momentary or stable oral colonizers.

In the present study only the effect of short term administration of probiotics was assessed. As this also resulted in significant reduction of plaque and gingival status it seems plausible that prolonged administration of probiotic preparations may have a preventive role against development of plaque and gingivitis.

The subjects selected in this study were 15-16 years age group, which was important for the present study, for the assessment of periodontal disease indicators in adolescents[18].This age group is considered to markperiodontal manifestations related to endogenous sex hormones[19]. Puberty marks initiation of changes from maturation into adulthood[20]. Several cross-sectional and longitudinal studies[21, 22, 23] have demonstrated an increase in gingival inflammation without accompanying an increase in plaque levels during puberty. Both estradiol and progesterone have been shown toselectively accumulate by *P.intermedia* as a substitute for vitamin K, and thus postulated to be acting as a growth factor for this microorganism.[24]

Another reason for selecting this age group was the intellectual ability of the child. In accordance with Jean Piaget at the age of seven years a child largely corresponds to an increase in cognitive development where by the child develops a sense of semi- logical reasoning to infer physical cause- effect relation-nships.Thus in this age group a positive compliance could be expected from a child. To our knowledge only one study reported use of oral probiotic in the age group between 7-14 years[5].

V. Conclusion

Probiotic therapies, once discussed primarily in the context of "complementary" or "integrative" medicine, are entering the therapeutic mainstream in maintaining the oral health. This concept prompts a new Horizon on use of probiotic mouthrinse in reduction of plaque and gingivitis.

VI. Limitations

It is pertinent to highlight some limitations of this study in order to subsidize future clinical trials in this field as follows.

(i)Probiotic effects are strain-specific, thus each individual bacterial strain needs to be tested separately, and the effects described for one strain cannot be directly applied to others. Unfortunately, mislabelling of strains in probiotic products seems to be a common problem . On the other hand, multispecies or multistrain probiotic products can be even more effective than products with only one bacterial strain, making the scientific evaluation of the mechanisms of the probiotic activity even a more complicated task.

VII. Recommendations

Enlarging the duration of treatment may be an alternative to assess the effects of prolonged use on oral mucosa and teeth. In addition, since our findings have indicated a good safety pattern of the product in a 14-day regimen, long-term trials are now encouraged to check

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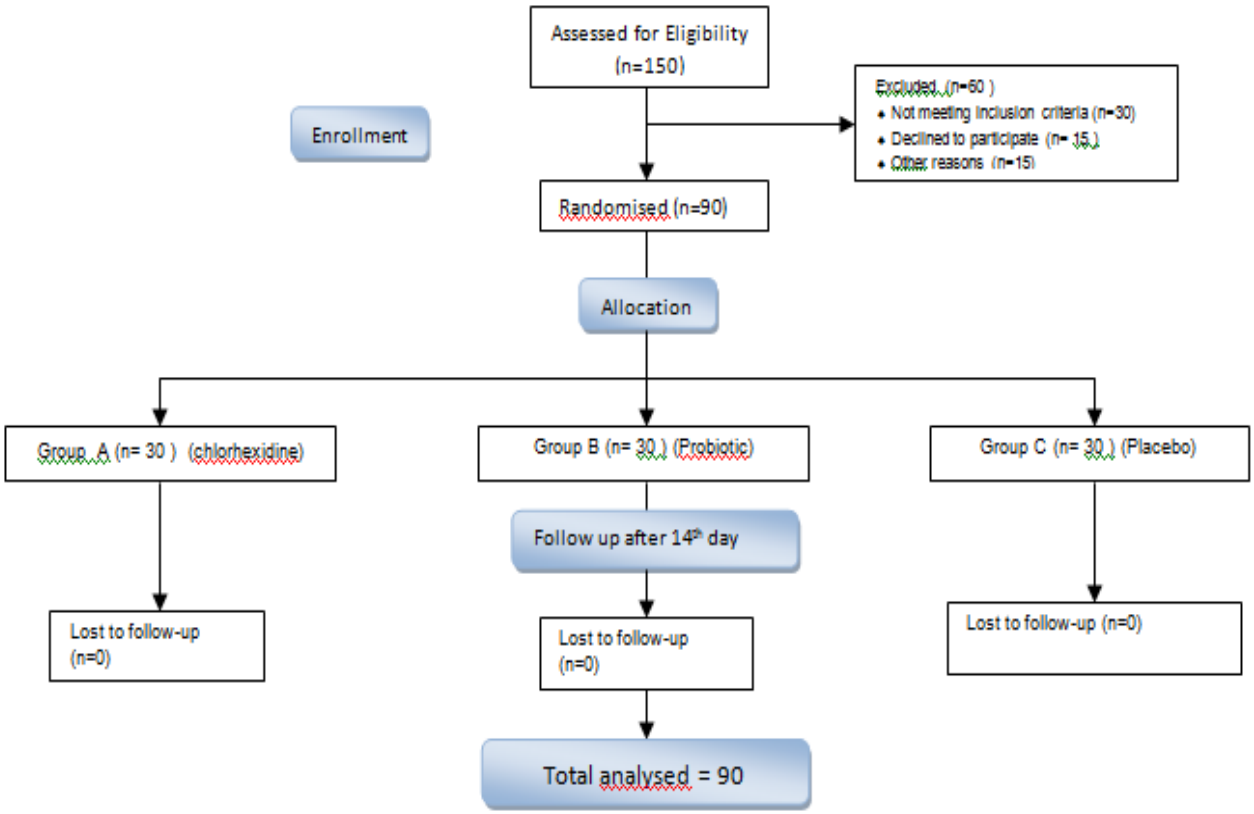
CONSORT FLOW DIAGRAM



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By Daniel Celli, Alessandro De Carlo, Enrico Gasperoni & Roberto Deli
University of the Sacred Heart, Italy

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Materials and Methods: The sample consisted of 31 patients with a pre-treatment mean age of 9.09 years. At the time of drawing up this manuscript, 14 patients of the entire sample reach the 3 years follow-up. The occlusal adjustment procedure was performed in centric relation. Every patient was treated in the second phase with fixed arch wires and finally a fixed lower retainer was placed. Pretreatment and posttreatment cephalometric changes were compared with dependent t tests.

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Strictly as per the compliance and regulations of:

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Conclusion: The selected sample showed a variable skeletal relationship except for a marked anterior open-bite which underwent this treatment procedure. Despite these odds, the findings of this study suggest that openbite treatment with occlusal adjustment provides statistically significant results and clinically good stability over time.

Keywords: open bite, orthodontic, orthodontic appli - ances, occlusal adjustment.

I. Introduction

Malocclusions characterized by anterior open bite are often difficult to treat successfully. Numerous theories have been proposed for aetiology of open bite, including heredity, unfavourable growth patterns, digit habits, enlarged lymphatic tissue function, health, and stability may occur with anterior open bite. These difficulties may include diminished dental aesthetics during speech and when smiling, lack of incisor guidance and canine disclusion, resulting in molar cuspal wear, exacerbation of temporomandibular dysfunction, lisping and involuntary spitting when speaking, posterior cross bite with functional shift of the mandible related to a posterior collapse of the maxilla, and maxillary incisor root resorption. [1,2,3]

Skeletal open bite is usually considered as a deviation in vertical relationship of the maxillary and mandibular dental arches with a lack of contact between opposing segments of teeth. If however, a dento-alveolar compensatory mechanism is involved, functi - onal occlusion can be reached.[4]

Therefore, orthodontic treatment consisted mainly of dento-alveolar changes and modification of oral habits. In case of unfavourable skeletal patterns, it could be necessary an orthognatic surgery correction.[5]

Unfortunately, many authors reported significant relapse of open bites treated either surgically or with orthodontic appliances. Skeletal changes greater than those observed in untreated adults have been noted beyond 1 year post-surgery in adult patients who had surgical correction of a long face deformity. [6,7]

Occlusal adjustment for the correction of anterior open bite is a therapeutic method already described and not very widespread in the literature. Few cases have been reported recently with an occlusal vertical correction, by grinding, along orthodontic therapy. Janson, Crepaldi et al. [2008] reported change in the occlusion, function and dentin sensitivity by occlusal adjustment on permanent teeth. Spena and

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[1] Greenlee GM, Huang GJ, Chen SS, Chen J, Koepsell T, Hujoel P. Stability of treatment for anterior open-bite malocclusion: a meta-analysis. Am J Orthod Dentofacial Orthop. 2011 Feb;139(2):154-69

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[7] Bondemark L, Holm AK, Hansen K, Axelsson S, Mohlin B, Brattstrom V, Paulin G, Pietila T. Long-term stability of orthodontic treatment and patient satisfaction. A systematic review. Angle Orthod. 2007 Jan;77(1):181-91

Gracchus [2008] reported a vertical progressive reduction of the deciduous teeth with braces and functional exercises.

The aim of the present study was to evaluate the effect of deciduous teeth grinding during mixed dentition in the occlusion, by the control of permanent molars eruption, using contemporary maxillary expansion appliance and palatal grid to block the action of tongue muscles on dentoalveolar remodeling. Statistical analyses of the results and a 3 years follow-up were showed.

II. Material and Methods

A sample consisted of 31 patients (15 male, 16 female), was obtained from the files achieved during private practice in Pescara, Italy. All patients originally had an anterior open-bite malocclusion, with pre-treatment mean age of 9.09 years (SD 1.37, range 7 - 12). The mean age at the end of the treatment was 12.68 years (SD 1.58, range 11 – 15.5). All the patients were scheduled for a midterm follow-up at 3 years after treatment. At the time of drawing up this manuscript, 14 patients of the entire sample reached the 3 years follow-up. The occlusal adjustment procedure was performed in centric relation, according to the method of Okeson.[8] All patients signed an informed consent for the orthodontics treatment and the necessary follow-up.

a) Cephalometric Analysis

All cephalometric radiographs were realized at pre-treatment (T1), immediately post-treatment (T2), and after three year (T3). They were digitized in double-blind by two primary authors, using TopCeph® software analysis. The cephalograms were then verified for landmarks location and anatomic contours in order to eliminate any casual errors by the operators. All cephalometric easurements are described in Table I.

Table I : Cephalometric values used to evaluate the samples

Cephalometric measurements	
SNA	Angle between lines S-N and N-B
SNB	Angle between lines S-N and N-B
ANB	Angle between lines N-A and N-B
Wits	Distance between perpendicular projections of Points A and B on the functional occlusal plane
FMA	Frankfort mandibular plane angle: angle between lines Po-Or and Go-Me
Sn.GoGn	Angle between lines S-N and Go-Gn
Sn.anspns	Angle between lines S-N and ans-pns (maxillary plane)
anspns.GoGn	
Go geometric	Angle between posterior (Go-Ar) and lower borders (Go-Me) of the lower jaw
(+1)/anspns	Angle between long axis of upper incisor and ans-pns
(-1)/GoGn	Angle between long axis of lower incisor and Go-Gn
Overjet	Distance between incisal edges of maxillary and mandibular central incisors, parallel to Frankfort plane
Overbite	Distance between incisal edges of maxillary and mandibular central incisors, perpendicular to occlusal plane
Interincisal angle	*Angle* formed by long axis of the upper incisor and long axis of the lower incisor

Any disagreements were solved by retracing the landmark or structure to the mutual satisfaction of both operators.

b) Inclusion Criteria

In 31 patients presented to clinic observation the initial examination revealed an 100% of anterior open bite, a 62% of mouth breathing, 55% of muscle deficit and 70% of lip incompetence at rest. The sample showed different skeletal relationship: 33.3% class III, 26.7% class II and 40% class I. The patient's long-standing tongue-thrust habit had contributed to an anterior open-bite up to - 7 mm and an over-jet up to 9 mm. The patient maintained good oral hygiene and showed no evidence of periodontal disease.

c) statistical analyses

All data were entered into spreadsheet Office Excel 2007 (Microsoft Corporation. Redmont, Washington State) and processed to calculate the mean, median, standard deviation, minimum value, maximum value. To compare the pre-treatment, post-treatment cephalometric changes, dependent t tests were used. The follow up cephalometric values were compared only among the small sample size (n=14) for the post-treatment and 3 years after treatment. The level of significance was 5%. These analyses were performed with PhStat 2 software (statistic add-in Microsoft Excel, version 2.7, Prentice Hall, Inc., Pearson Education).

[8] McNamara JA Jr, Seligman DA, Okeson JP. Occlusion, Orthodontic treatment, and temporomandibular disorders: a review. J Orofac Pain. 1995 Winter;9(1):73-90. Review

III. Treatment Plan

Treatment objectives aim to correct the anterior open bite and achieve ideal overbite and overjet, correct the transversal discrepancy of the two dental arches increasing the space for the future permanent teeth, and achieve a correct Class I dental relationship.

A rapid palatal expander HYRAX type is inserted between the second deciduous molars and canines. The active arms of the appliance are extended to the canines, embraced them, with cannulas for the insertion of a lingual grid at the end of the activation.

During the period of RME treatment, the deciduos molars are ground with a diamond bur, to anticipate and control the contemporary eruption of the permanent first molars, and thus, the vertical dimension.(Fig.1)

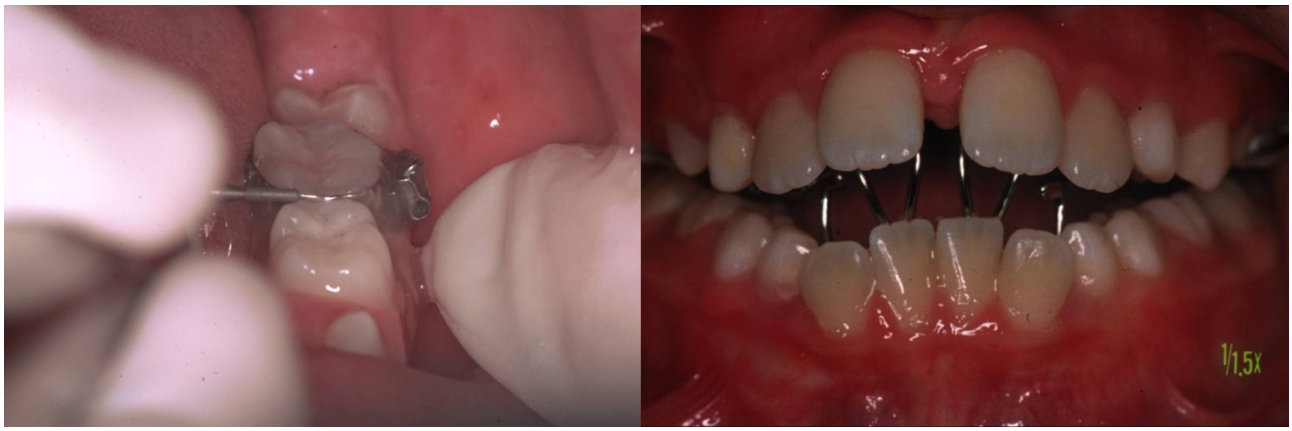

Figure 1 : Occlusal grinding of the deciduos teeth and Rapid maxillary expansion during treatment

The expansion time was 3.4 to 4 weeks. A lingual grid was positioned just after the rapid maxillary expansion to prevent the wrong lower tongue posture and allow subsequently the setting up of an oral seal during deglutition. The rapid maxillary expansion was kept for six months of retention.

IV. Treatment Progress

After the treatment was suspended, maxillary and mandibular arches were bonded with an HSDC (hybrid system Daniel Celli), an hybrid straightwire appliance: anterior conventional brackets (STEP, Leone® s.p.a, Florence, Italy) and posterior passive self ligating brackets (F1000, Leone® s.p.a, Florence, Italy). If necessary strategic brackets positioning was used. A .014" or 016" nickel titanium main archwire was placed for initial alignment depending on the degree of dental crowding. Over the next seven/nine months, the arch - wires were generally stepped up to .016x.025 HANT, .019" x .025" HANT (heat activated nichel titanium), .020" Australian stainless steel, .019" x .025" stainless steel wire. Intermaxillary elastics were used with the last leveling arch wire to correct the occlusion and the dental open bite. The case was finished with a sectional arch wire .019" x .025" stainless steel on the maxillary arch and a .016" Ni-Ti on the lower arch, with intermaxillary elastics worn to maintain the correction. (Fig. 2)

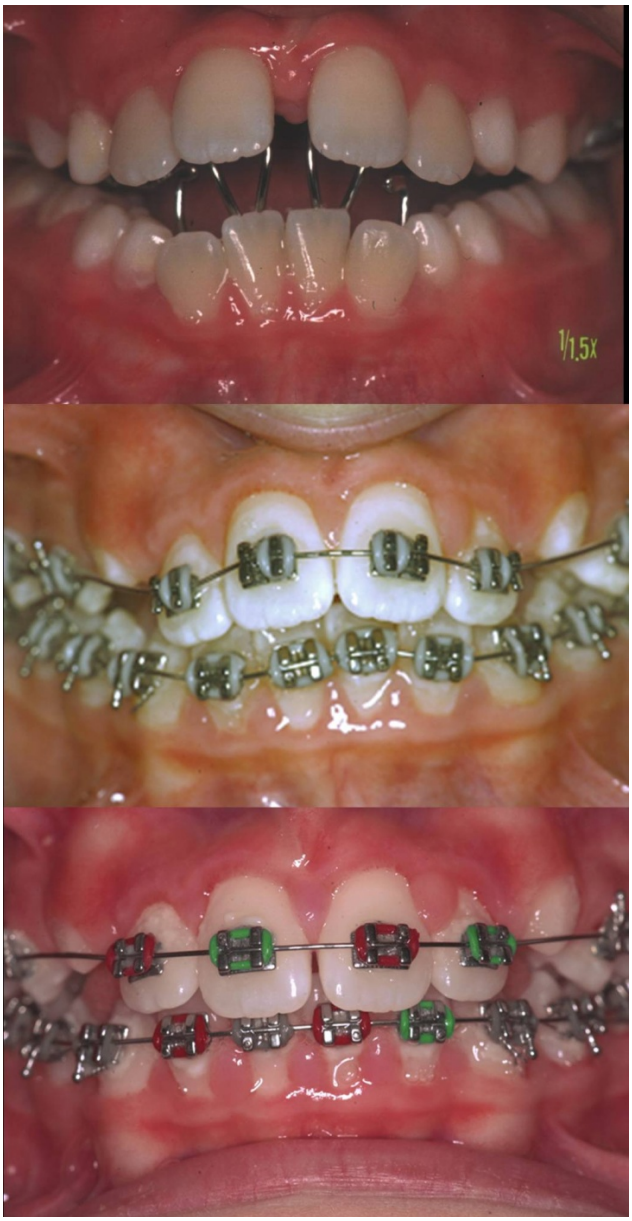

Figure 2 : Treatment progress with nickel titanium main archwires and anterior open bite closing during the treatment

V. Results

At the end of active treatment, the brackets were debonded, and a fixed retainer was placed on the lower anterior arch, while a wraparound removable retainer, for the upper dental arch was delivered. The mean time of active treatment was 3.22 years (SD, 0.93). Post-treatment facial and intraoral photographs showed good aesthetic, skeletal balance and good functional results in all the patients. Final occlusal results, dento-alveolar compensation and root angulations were acceptable, with an adequate overjet and overbite. Superimposition of pre- and post-treatment cephal - ometric tracings, showed an advancement of A-point and ANS towards an anterior-lower direction.

The pre-treatment overbite had a mean value of -2.5 mm (SD - 2.22, median – 2, range -7 to 0) while post-treatment overbite had a mean of 1.75 mm (SD 1.75, median 1.75, range 0 to 3). Overbite increased significantly with treatment and caused significant changes in other skeletal and dentoalveolar variables. In fact there was a mean increase in overbite after the therapeutic protocol used of 4.25 mm (SD 2.58, median 3.5, range -0.5 to 7.5) The dependent t test analysis confirmed the statistically significant of the results sho - wed (t test 0,000137, $P < 0.05$). There was a statistically significant increase in other dentoalveolar cephalometric values (-1/GoGn; interincisal angle); the vertical facial pattern variables, specially the maxillary plane (SN/anspns; anspns/GoGn), had statistically significant reductions. Starting from the dentoalveolar pattern, the pre-treatment -1/GoGn changed from an average of 95.00 (SD 9.64, Median 93, range 76 to 114) to an average of 91.63 (SD 9.54, Median 94, range 70 to 104). The pre-treatment interincisal angle mean was 118.5 (SD 11.689, Median 121, range 98 to 138) while the mean value after treatment was 123.5 (SD 8.691, Median 124.5, range 116 to 145) (Table II). Upon the whole sample, 30 patients had a positive overbite after the therapeutic protocol whereas 1 had a negative overbite (3.23% of the patients) due to the lack of patient cooperation and therefore, he was excluded from the statistical analysis. The mean changes of the other variables and their standard deviations are also shown on Table III. After 3 years of follow-up, the sample (n=14) showed minimal changes in cephalometric values chosen, as confirmed by the statistical results shown on Table IV. Of the 14 patients treated, five repo-rted almost the same values as at the end of treatment.

Table II : Cephalometric results before and after treatment calculated by the Mean, Median and Standard Deviation (sample n=30)

	Before Treatment			After Treatment		
Cephalometric measurements	Mean	Median	SD	Mean	Median	SD
SNA	81,141	81	4,063	81,090	81	4,437
SNB	77,883	77,75	3,892	78,272	77	4,557
ANB	3,266	4	2,237	2,818	3	1,453
Wits	-2,091	-2,05	3,368	-2,163	-2,5	2,806
FMA	29,85	29	5,139	31	31	5,196
Sn.GoGn	38,641	39,35	5,989	38,272	38	5,178
Sn.anspns	6,716	8,5	3,461	8,6	10	3,388
anspns.GoGn	32,083	31,5	4,010	30,6	31	4,753
Go	132,5	131	5,435	131,273	128	6,679
(+1)/anspns	113,167	112,5	6,912	113,545	115	3,939
(-1)/GoGn	95,008	93	9,644	91,636	94	9,542
Overjet	3,291	3,5	2,879	2,863	3	1,266
Overbite	-2,5	-2	2,225	1,75	1,75	1,138
Interincisal angle	118,5	121	11,689	123,5	124,5	8,691

Table III : Cephalometric changes before and after treatment and results of dependent t tests (P< 0.05)

	Change before-after			
Cephalometric measurements	Mean	Median	SD	*P*
SNA	0,05	0	0,373	0,6778
SNB	0,389	0,75	0,664	0,37941
ANB	0,448	1	0,783	0,58042
Wits	0,071	0,45	0,562	0,87619
FMA	1,15	2	0,056	0,35907
Sn.GoGn	0,368	1,35	0,81	0,40987
Sn.anspns	1,883	1,5	0,073	0,02875*
Anspns.GoGn	1,483	0,5	0,743	0,0483*
Go	1,227	3	1,244	0,63869
(+1)/anspns	0,378	2,5	2,972	0,81503
(-1)/GoGn	3,371	1	0,102	0,03909*
Overjet	0,428	0,5	1,61	0,45294
Overbite	4,25	3,5	2,58	0,000137*
Interincisal angle	5	3,5	2,997	0,04786*

Table IV : Cephalometric results at the end of the treatment and after 3 years of follow-up (sample n=14)

	After Treatment			Follow-up 3 years		
Cephalometric measurements	Mean	Median	SD	Mean	Median	SD
SNA	77	77	1,632	77,25	77,5	1,707
SNB	74,625	74,75	2,286	74,75	75	2,217
ANB	2,375	2,5	1,108	2,5	2,5	1,29
Wits	-2,95	-3,4	2,23	-2,25	-2	2,217
FMA	28,75	29	2,986	28,175	27,85	3,374
Sn.GoGn	40	39,5	3,162	39,05	38,5	4,18
Sn.anspns	11,525	10,8	2,025	11,25	11,5	0,957
anspns.GoGn	29,15	29,8	3,875	29,1	29,2	3,583
Go	130,5	130,5	5,8	130	130	5,77
(+1)/anspns	115,375	116,5	2,625	115,75	116,5	2,629
(-1)/GoGn	94,25	94,5	1,707	95,975	96	1,862
Overjet	2,75	3	1,258	2,75	3	1,258
Overbite	1	0,75	0,707	1,2	1,15	0,62
Interincisal angle	118	117	5,887	118	117	4,031

VI. Discussion

The treatment protocol described for the open bite correction, is composed of a combination of progressive vertical reduction of the deciduous first and second molars and fixed appliance that requires minimal patient's compliance.

From the analysis of the treated patients, in almost all of them the therapeutic objectives were reached. The selected sample (n=30) showed a variable skeletal relationship except for a marked anterior open-bite that has been underwent this treatment procedure. Although these odds, related to subsequent different cephalometric sagittal markers, they can be considered indicative of the significant scientific value of the tested protocol that can be applied in any different subjects and clinical situations.

Rapid maxillary expansion (RME) is a universally employed technique for correction of posterior crossbites and gain in arch perimeter in patients with tooth-size/arch-size discrepancies, like skeletal Class II and Class III. [9] The device leads mainly skeletal and alveolar volume variations of the palate, with orthopedic effect of rapid expansion, and subsequently in selected cases, antero-posterior and vertical mandibular changes in skeletal Class II patients.[10]

Unfortunately a slight relapse occurs after device removal in long term, the greates being in intercanine width.[11]Mainly expansion stability could be due to three factors: young age of the patients, which led to a good orthopedic result, prolonged retention period, which permitted complete remineralization of the palatine suture, and repositioning of the tongue within the arches following an increase in upper diameter.[12]

Rapid maxillary expansion is an important treatment factor related to the open bite correction. It is also associated with a significant increment in nasal volumes and in the transverse diameter of the maxilla, with statistically significant increase respectively in decongested total nasal volumes and in binasal cavity. Regard to breathing posture, the role of this procedure still remains debatable.[13]

The anterior tongue rest posture plays an etiologic role in the relapse of anterior open-bite. [14]The open bite reduction and its stability can also be attributed to the tongue spurs, which interfere with the wrong lower tongue posture and with the establishment of an oral seal during deglutition.[15]

The effectiveness of the tongue spurs has been repeatedly the subject of criticism and literature review. Its effect changes, depending on various parameters

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such as length of spurs use, age of stakeholders, the skeletal class and function, design spurs. The tongue spurs force a change on the anterior tongue rest posture, which in turn allows incisors to erupt, closing the anterior open bite.[16]

The authors decided to perform occlusal adjustment only on deciduous molars. Grinding is an aggressive procedure for the dental tissues, with permanent effects on teeth. Working on deciduous teeth becomes a transitional and non-invasive procedure for the patient. The results confirmed previous studies demonstrating the efficacy of the procedure to close an open-bite.[17,18] Selective grinding, to be effective, must be achieved during the period of growth and, namely, at the moment of maxillary and mandibular permanent teeth eruption. A loss of occlusal contact between the upper and lower molars resulted at this time. The deciduous teeth will be ground up to that a physical contact would be re-establish with the antagonist molars.

The proper management of an open-bite patient is based on the choice of a therapeutic protocol that takes into account the difficulties and long-term stability of this treatment. Early treatment of open bite allows compensatory craniofacial growth and reduces the need for a second phase of treatment that might involve extractions or orthognathic surgery. When the open bite correction begins in deciduous or mixed dentition, as in the treatment protocol proposed, the appliances could be very effective and produce faster response in younger subjects.[19] Finally, the use of multiple therapeutic options allows us to get satisfactory and stable results over time, as demonstrated by the 3-years follow up.

Accordingly, it is important to notice the cephalometric changes after the therapeutic protocol. There was a statistically significant decrease of the facial pattern angles as well as the dento-alveolar terms. Ans-Pns plane rotate clockwise in the mid-sagittal plane. The upper and lower incisors change their position in order to close the bite; therefore a good aesthetic condition and the protection of incisors guidance during protrusion are kept over time.

Although after 3 years the study presents a small sample, it's already possible to identify a stable results, especially for five patients showing almost any change at the follow-up (Fig 3). It was interesting and relevant that larger over jets and tooth display (at rest) disappeared and got a natural harmony to patient's face.

[16] Meyer-Marcotty P, Kochel J, Stellzig-Eisenhauer A. The impact of spur therapy in dentoalveolar open bite. Aust Orthod J. 2013 Nov;29(2):145-52

[17] Janson G, Crepaldi MV, de Freitas KM, de Freitas MR, Janson W. Evaluation of anterior open-bite treatment with occlusal adjustment. Am J Orthod Dentofacial Orthop. 2008 Jul;134(1):10-2

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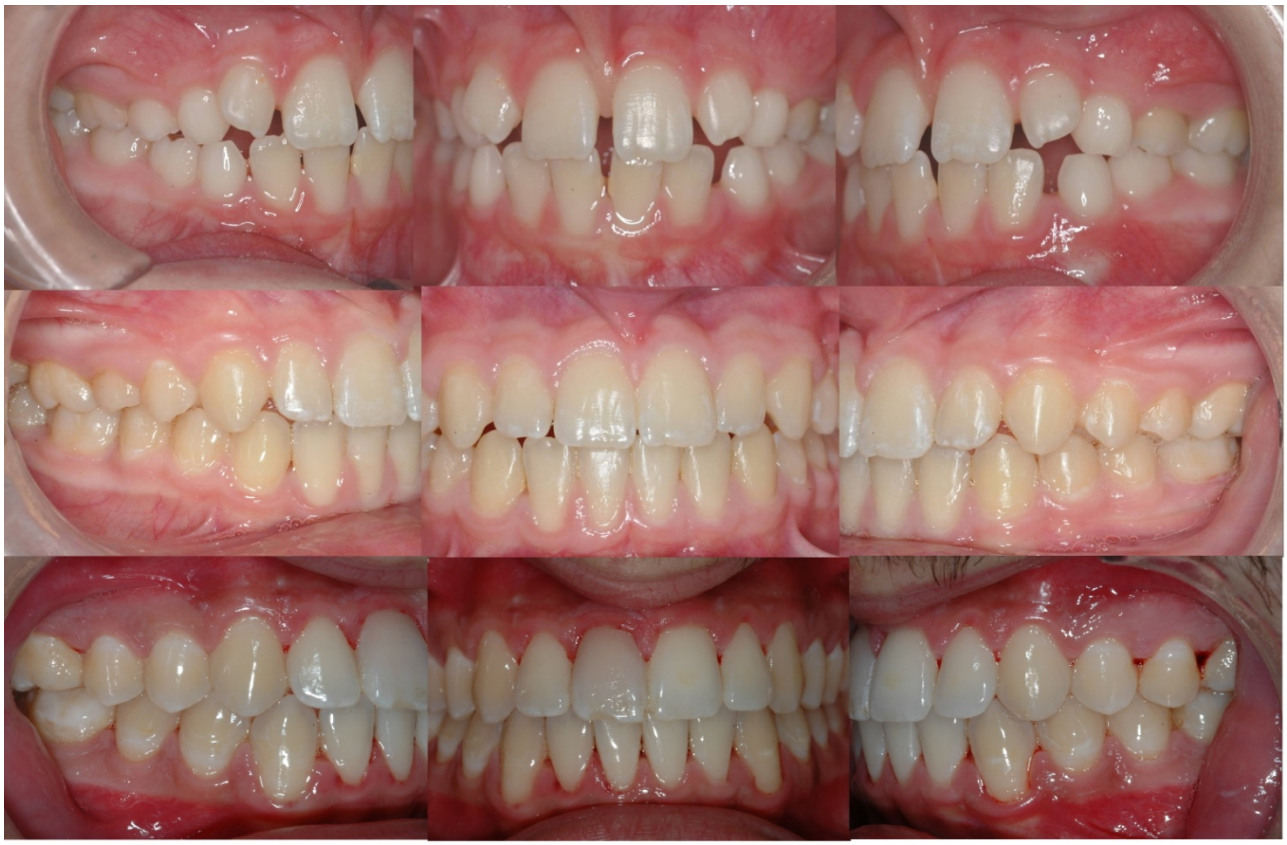

Figure 3 : Intra oral photographs before treatment, at the end of treatment and after 3 years

VII. Conclusion

This study suggests that selective grinding of deciduous teeth permits to obtain fast therapeutic results with harmless and transitory effects for dental tissue. Its action, coupled with the rapid expansion of the palate and tongue spurs, allows the closure of open bite, followed by orthodontics. The early treatment proposed of open-bite tendency results in a rapid control of the vertical dimension, in a significant and stable improvement of a correct and functional occlusion and in perceived facial aesthetic.

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Nonsurgical Management of Cutaneous Sinus Tract of Odontogenic Origin: A Case Report

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Abstract- Draining cutaneous sinus tracts in the area of face may be caused by chronic dental infections. Misdiagnosis of these cutaneous sinus tracts usually leads to destructive invasive treatment of the skin lesions that is not curative. Diagnosis of the cause may be challenging but is the key to successful therapy. Successful repair depends primarily on removal of etiological factors. This scientific paper aims to present a case, treated successfully & closure of the tract achieved by conventional root canal therapy.

Keywords: cutaneous sinus, odontogenic infection, sinus tract, trauma.

GJMR-J Classification: NLMC Code: WO 460

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Nonsurgical Management of Cutaneous Sinus Tract of Odontogenic Origin: A Case Report

Dr. Gautam P. Badole [α], Dr. Rajesh Kubde [σ], Dr. Mohit Gunwal [ρ] & Dr. Shital G. Badole [ω]

Abstract- **Draining cutaneous sinus tracts in the area of face may be caused by chronic dental infections. Misdiagnosis of these cutaneous sinus tracts usually leads to destructive invasive treatment of the skin lesions that is not curative. Diagnosis of the cause may be challenging but is the key to successful therapy. Successful repair depends primarily on removal of etiological factors. This scientific paper aims to present a case, treated successfully & closure of the tract achieved by conventional root canal therapy.**

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I. Introduction

A chronic inflammation of pulpal origin is one of the reasons for an extra oral sinus of odontogenic origin.[1] The discharge of purulent exudates usually is associated with periapical radiolucent area and goes through tissues and structures along the path of least resistance.[2] Cutaneous lesion may develop over a long period of time and is often distant from the site of primary infection. Hence successful management of these odontogenic cutaneous sinus tracts of pulpal pathology depend on proper diagnosis.[3,4] The site of drainage depends on the tooth which is diseased, and the apex position relatively to muscular attachments, bacterial virulence and lower host resistance.[5] If the apices of the teeth are above the maxillary muscle attachments and below the mandibular muscle attachments the spread of infection may be extraoral.[6] These sinus tracts occurring more frequently from infected mandibular teeth than from infected maxillary teeth and particularly on the chin or in the submandibular area.[7] The involved tooth is asymptomatic as there is absence of swelling or pain results from pressure build up.[7] Patient first visits a physician for evaluation and treatment. Diagnosis of cutaneous sinus tracts of dental origin presents constant challenge to practitioners.[8, 9] Based on literature reports, misdiagnosis has often worsened the chronicity of the lesion and has pronounced effects on facial aesthetics due to unnecessary treatments such as multiple biopsies, antibiotic regimens resulting in further skin scarring.[10] It has been observed that systemic antibiotic therapy will result in a temporary reduction of the drainage and apparent healing. Root canal therapy in minimal scarring of skin.[11] The purpose of this report is to present a case of cutaneous sinus tract successfully managed with only conventional root canal treatment

II. Case Report

A 17 year-old female patient was referred to the Department of conservative dentistry and endodontics with a complaint of extra oral nodulous growth on her chin for the past five months. Patient had given a history of treatment for the same nodule from dermatologist since its appearance but not subsided. On extraoral examination, 0.5×0.5 cm nodule like lesion (Figure 1) was present on her chin. Palpation elicited nontender nodule and fixation of the lesion to underlying bone. Intraoral examination revealed no mucosal lesions or buccal sulcus swelling but elicited tenderness at the root apex of 31. Pulp vitality showed negative response with 31. Periapical radiograph of mandibular left central incisor revealed periapical radiolucency (Figure 2). While recording the dental history patient revealed that she met with trauma two years ago, but there was no pain present with any of the tooth. On clinical and radiographic examination, chronic apical periodontitis with 31 leading to extraoral sinus tract was made. Root canal treatment was planned with 31.

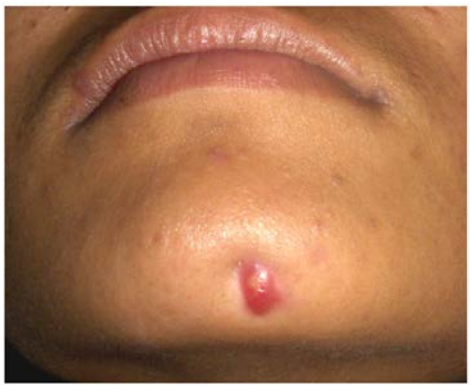

Figure 1 : Extra-oral sinus on chin

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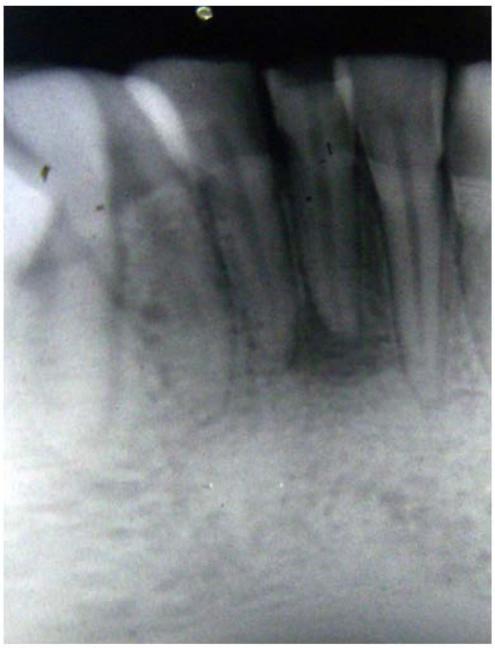

Figure 2 : Periapical radiolucency with 31

Under the rubber dam isolation, access opening was done with 31 and samples were collected for both aerobic and anaerobic culture so that effective antibiotic treatment was started. Working length was determined with apex locator (Root ZX; Morita, Tokyo, Japan.) and confirmed with intra-oral periapical radiograph. Cleaning and shaping was completed with step-back technique using hand K-files (Dentsply Maillefer, Ballaigues, Switzerland). Chlorhexidine (Endo-CHX, Prime Dental Product, Mumbai, India.) was used as intracanal irrigant and not sodium hypochlorite, duo the risk of apical extrusion of the irrigant. Canal was dried with paper points and calcium hydroxide (RC Cal; Prime Dental Products, Thane, India.) was placed within the canal as intracanal medicament. After a week obturation was completed using gutta-percha points with cold lateral condensation technique (Figure 3). Patient was evaluated for a period of 3 months showed complete healing of extraoral sinus (Figure 4). Intra oral periapical radiograph showed complete healing of periapical radiolucency (Figure 5).

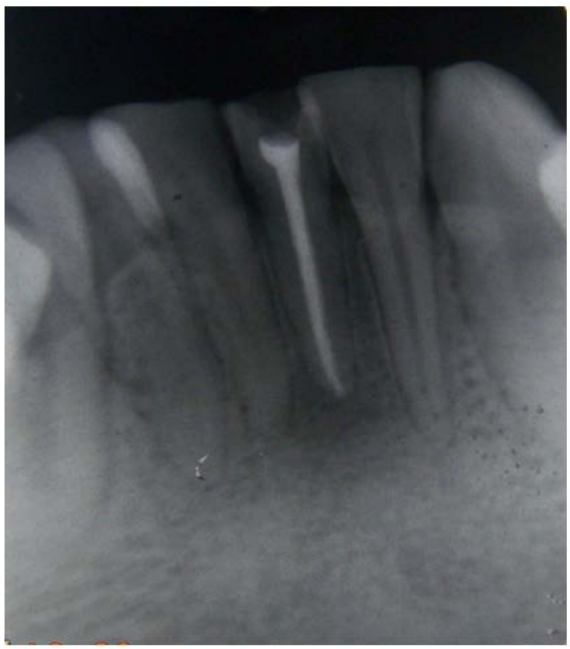

Figure 3 : Post-obturation radiograph

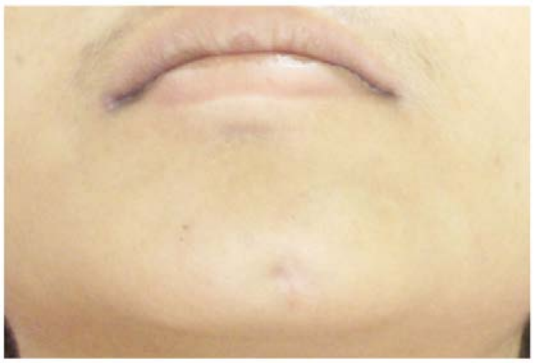

Figure 4 : Complete healing of extra-oral sinus

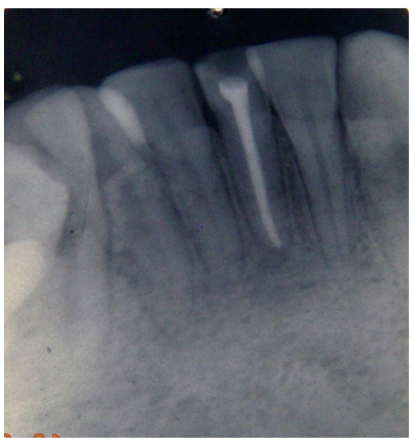

Figure 5 : Healing of periapical lesion on radiograph

III. Discussion

The cutaneous sinus tract of dental origin is an uncommon but well documented condition in the

medical, dental, and dermatological literature.[6] Cutaneous dental sinus or Odontogenic cutaneous sinus tract or Cutaneous facial sinus tract of dental origin was first stated by Winstock in 1950.[12] Approximately 80% of these sinuses related to mandibular teeth and 20% with maxillary teeth.[8] The characteristic lesion is erythematous, smooth, symmetrical nodule, 1-20 mm in diameter. There is periodic drainage and crusting in some cases and the lesion is depressed below the normal skin surface. A cord - like tract can be felt attached to the underlying bone.[8] If the sinus tract is patient a lacrimal probe or gutta-percha cone can be introduce into the opening and confirmed the affected tooth on intraoral periapical radiograph. Pulp vitality test should perform on suspected as well as on adjacent teeth. Culture and sensitivity testing of discharged fluid should be performed to rule out fungal and syphilitic infection.[13]

The clinical differential diagnosis of cutaneous draining sinus tracts includes osteomyelitis, congenital fistula, salivary gland fistula and infected cyst and deep mycotic infection. In addition, skin lesions such as pustules, furuncles, foreign-body lesions, squamous cell carcinoma and granulomatous disorders show the similar superficial appearance.[4]

Nonsurgical endodontic therapy is the treatment of choice if the tooth is restorable or extraction if nonrestorable.[11] There are different opinions regarding the removal of sinus tract, some authors recommended excision of cutaneous lesion and sinus in continuity at the time of treatment of the dental pathology with immediate plastic repair of cutaneous site.[12, 14] Other believes that on removal of primary odontogenic cause cutaneous lesion heals without any intervention within 5 to 14 days,[3] dimpling and hyperpigmentation of area occurs which fade over time and a surgical revision of bigger scar might be needed to provide better cosmetic result in future.[3, 15]

IV. Conclusion

The key to a successful treatment of cutaneous sinus of dental origine must lay in healthy communication between the dentist and the physician in order to provide for timely recognition and treatment of such cases. Basic principles of root canal treatment should be used judiciously to create a favourable environment while effectively eliminating the pathogens and giving the body's immune, healing and repair mechanism a chance to achieve the desired result.

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Correlation between Estrogen Deficiency and Chronic Desquamative Gingivitis in Female Patients

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Abstract- The oral mucosa may be affected by a variety of systemic diseases and oral lesions most often may precede several mucocutaneous or systemic disorders. The systemic basis for many of the oral lesions is not clearly known. One such oral disease which may have a strong systemic basis for its pathogenesis is chronic desquamative gingivitis (CDG). In the literature there are conflicting reports as to the mechanism of pathogenesis of this clinical entity. Some investigators consider this as a unique clinical disease, whereas, others consider it as the gingival manifestation of disease processes having a strong correlation with the fluctuation of female sex hormones. This study was conducted to find out a correlation between circulating levels of serum estrogen (the female sex hormone) and occurrence of CDG in female patients.

Keywords: chronic desquamative gingivitis, estrogen, hormone replacement therapy, gingivitis.

GJMR-J Classification: NLMC Code: WU 300

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Correlation between Estrogen Deficiency and Chronic Desquamative Gingivitis in Female Patients

Pramod John

Abstract- The oral mucosa may be affected by a variety of systemic diseases and oral lesions most often may precede several mucocutaneous or systemic disorders. The systemic basis for many of the oral lesions is not clearly known. One such oral disease which may have a strong systemic basis for its pathogenesis is chronic desquamative gingivitis (CDG). In the literature there are conflicting reports as to the mechanism of pathogenesis of this clinical entity. Some investigators consider this as a unique clinical disease, whereas, others consider it as the gingival manifestation of disease processes having a strong correlation with the fluctuation of female sex hormones. This study was conducted to find out a correlation between circulating levels of serum estrogen (the female sex hormone) and occurrence of CDG in female patients.

Keywords: chronic desquamative gingivitis, estrogen, hormone replacement therapy, gingivitis.

I. Introduction

As a disease entity, chronic desquamative gingivitis was first described by Tomes and Tomes in 1894. However, the term 'desquamative gingivitis' was first introduced by Prinz in 1932 for the presence of erythema, desquamation, erosion and blistering of attached and marginal gingiva.[1] Glickman and Smulow[3] stated that it is a clinical manifestation of several disorders. This is further confirmed recently by many other investigators. CDG is a clinically relevant entity as it can affect the oral health and is mainly mediated by certain hormonal deficiency states. Its clinical appearance is not significantly altered by traditional oral hygiene measures or conventional periodontal therapy. It is a fairly common complaint typically seen in females who are middle-aged or older[4, 5]. Many cases have also been reported in younger women, often associated with fluctuations in the circulating sex hormone levels.

CDG is a clinical condition with unclear and uncertain etiology. It is not a specific diagnosis but a descriptive term for non-specific gingival manifestation associated with different diseases.[6] It is not a disease but represents a reaction pattern of the gingiva which conceals other pathological processes. Some investigators consider it as a specific disease, whereas, others consider it as a manifestation of immunologically mediated mucocutaneous disorder which is aggravated by local plaque accumulation and chronic irritation, or a manifestation of a number of disorders ranging from vesiculobullous diseases such as cicatricial and bullous pemphigoid, pemphigus vulgaris, erosive lichen planus, erythema multiforme, psoriasis and allergy, to adverse reaction to a variety of chemicals and allergens or manifestation of metabolic and hormonal disturbances. Though the investigators are confused about the etiology of CDG, many are of the opinion that there is a strong hormonal basis for the etiology of this condition.

It has been found that estrogen ointments when applied topically is effective in controlling this disease.[7] Yet another therapeutic measure recommended in certain refractory cases of desquamative gingivitis is hormone replacement therapy (HRT) with low dose estrogen. However, this should be done under the careful supervision of a physician or gynaecologist. A number of studies have shown that hormone replacement therapy (HRT) with estrogen can relieve the oral discomfort in post-menopausal women, thus establishing the role of female sex hormones in the healthy maintenance of the oral tissues.[6,7]

Estrogen is produced primarily in the ovaries. Some quantity of estrogen is also produced by the adrenal glands. Estrogen belongs to the category of sex steroid hormones and is a derivative of cholesterol and consists of a combination of three rings of six carbon atoms each (phenanthrene) and one ring of five carbon atoms (cyclopentane) to form a complex hydrogenated cyclopentanoperhydrophenanthrene ring system. Signals for estrogen production originate in the pituitary gland and the levels vary throughout life depending on the stage of a woman's menstrual cycle. The three major naturally occurring estrogens in women are est - rone (E1), estradiol (E2 or 17 β- estradiol or estradiol), and estriol (E3). Estradiol is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circul - ating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Though estriol is the most plentiful of the three estrogens it is also the weakest, whereas estradiol is the strongest.

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II. Review of Literature

Richman, Abarbanel[8, 9], as early as 1943 realized the significance of the female sex hormone, estrogen, in the maintenance of gingival health and had used exogenous estrogen preparations to successfully treat desquamative lesions of the gingiva. They perceived that estrogens increased epithelial kera - tinization and stimulated proliferation of the epithelial cells.

Daniel, E, Ziskin and Zegarelli, EV[10] in 1945 analyzed twelve patients, belonging to the age group of 21 to 67 years. According to them, the disease is hypothetically designated as a local manifestation of a metabolic disturbance. Various causes of this disturbed metabolism were also considered such as abnormal functioning of the thyroid gland and the interrelationship of the vitamins and estrogens. Their data suggested that a local depletion of estrogen in the oral tissues may play a major causative role. Estrogen ointments applied topically were found to be effective in controlling the disease.

Milton B. Engel et al. in 1950[11] had studied the pathogenesis of desquamative gingivitis and stated that the boundary between the epithelium and the connective tissue of the gingiva is formed by an optically homogeneous ground substance, which, together with the embedded fibres is termed the basement mem - brane. The major component of the homogeneous ground substance is an insoluble carbohydrate-protein complex which is thought to be highly polymerized. Although relatively resistant to chemical treatment, it may exhibit lability in certain physiologic and pathologic processes. The investigators are of the opinion that many of the disturbances of the gingiva originate in the connective tissue. In desquamative gingivitis, the slig - htest pressure of the finger or from an air blast causes a clean separation of the epithelial layer from the und - erlying connective tissue in an almost spontaneous manner. The gingiva is marked by many ulcerated and bleeding areas. There was degeneration of the epit - helium and edema and inflammation of the connective tissue. Histopathology revealed absence of basement membrane. There was increased quantity of water-soluble carbohydrate-containing substances formed due to the action of depolymerizing enzymes. A low level of estrogen might lie behind the symptoms in desquamative gingivitis as the enzymatic activity of the connective tissue is subject to hormonal influences.

Theresa Kindler in 1954 [12] first described the Kindler syndrome which is characterized by blistering of the skin, photosensitivity, and desquamation of the gingiva. It is a rare autosomal recessive genod - ermatosis. Mc Carthy F. P, et al. in 1960 [13] studied 40 cases of desquamative gingivitis over a period of 12 years and concluded that chronic desquamative gingivitis is actually a nonspecific manifestation of variety of systemic diseases. He also proposed an etiologic classification for desquamative gingivitis based on the causative factors associated with chronic desqamative gingivitis such as dermatoses, hormonal deficiencies, abnormal response to irritation, chronic infection, and idiopathic causes.

According to Glickman and Smulow in 1964, desquamative gingivitis is a disease which is primarily a degenerative process mainly affecting the gingiva. Löe in 1965 [14] reported that gingival inflammation and hyperplasia may be associated with hormonal changes taking place during puberty, menstruation and pregnancy. Kullander and Sonesson in 1965[15] had reported that many oral changes can occur as a result of a decline in the estrogen levels in women. They had reported many oral changes associated with menopause. Their investigations led to the conclusion that strong relationship exists between circulating hormonal levels and inflammatory changes of the oral mucosa.

Jenson et al. in 1968[16] and Gorksi et al. in 1968[17] observed that the sex steroid hormones bind to intracellular proteins with specificity and high affinity and this concept has led to the theory that steroid hormones act via the receptors to initiate biological responses. According to Kalkwarf in 1978[18] and Pankhurst et al. in 1981[19], based on their extensive studies, have concluded that gingival inflammation may be commonly seen in women taking oral contraceptive medication. The nature of this inflammatory response of the gingiva is similar to chronic desquamative gingivitis. Therefore, they are of the opinion that chronic desquamative gingivitis may be caused by oral contraceptive medication.

Menopause and its effects on the oral health has been extensively studied by Parvinen in 1984[20]. He is of the opinion that many oral diseases, including chronic desquamative gingivitis could be attributed to estrogen deficiency as in the case of post-menopausal state.

Green in1986[21] and Greene, et al. in 1986[22] have identified estrogen receptors (ER) in the gingiva. Later by some other investigators the mechanism of action of estrogen-estrogen receptor was studied which led to the identification estrogen subtypes. The classical estrogen receptor (ER) was renamed ER α after the identification of ER β.

Morishita et al. in 1988[23] suggested that unbalanced secretion of sex hormones, i.e. an increase of estradiol and a decrease of progesterone, might be one of the factors promoting gingivitis during puberty. However, the mechanisms of the effects of these hormones in the initiation of gingival inflammation are not clearly known. Masaharu Miyagi, et al. in 1992[24] reported a significant positive correlation between the concentration of progesterone in the plasma of females and the chemotactic ability of polymorphonuclear leuko-

cytes (PMN) in vitro. In males, there was no significant relationship between plasma levels of sex hormones and PMN chemotactic ability. Further sex hormones had no effect on the chemotaxis of monocytes. These results suggest that the altered PMN chemotaxis associated with gingival inflammation may be due to the effects of female sex hormones. They have also stated that the gingival inflammation is exaggerated during puberty and pregnancy. Altered levels of circulating sex hormones during puberty are considered to aggravate gingivitis induced by bacterial plaque. It is generally accepted that the bacterial plaque induces gingival inflammation through interactions with host defense mechanisms. In such defense mechanisms, phagocytic cells such as PMN leukocytes and macrophages are suggested to play an important role. Therefore, they hypothesized that sex hormones may cause inflammation by their actions on the functions of PMNs or monocytes.

Ciocca and Roig in 1995[25] reported the expression of RNA-m at the specific estrogen receptors by means of polymerase chain reaction (PCR) studies, through which it can be assessed whether the receptor is functional, that is whether there is genetic control or cell function control. Bonnie J. Deroo and Kenneth S. Korach in 2006[26] have reviewed estrogen receptors and human disease. They have mentioned that estrogen influences many physiological processes in human, not limited to reproduction. Estrogen is also implicated in the development or progression of numerous diseases. Estrogen mediates its effect through the estrogen receptor (ER), and plays a role in the development or severity of disease. According to them estrogens induce cellular changes through several different mechanisms. In the classical mechanism of estrogen action, estrogens diffuse into the cell and binds to a protein, the estrogen receptor which is located in the nucleus.

III. Materials and Methods

The study was conducted in the Department of Oral Medicine and Radiology, Amrita School of Dentistry, Cochin among female patients presenting with clinical signs and symptoms of chronic desquamative gingivitis and normal subjects (the control group).

Before carrying out the study, the institutional Ethical Committee approval was obtained. Among the 100 subjects selected for the study, 50 patients with clinical presentation of chronic desquamative gingivitis were taken as the study subjects (Group A or the study group) and the remaining 50 patients without CDG were taken as control subjects (Group B or the control group).

a) Inclusion Criteria

The following inclusion criteria were applied while selecting the subjects of Group A:

1. Patients with clinically diagnosable chronic desquamative gingivitis.
2. Patients with normal growth pattern and secondary sexual characteristics.
3. Patients should be free from any other endocrine disorders.
4. Patients should have had normal menstrual history (in case of post-menopausal women) and the patients should have regular menstrual cycle (in patients who have not attained menopause).
5. At least one year should have elapsed after the last delivery.
6. One week should have elapsed after the last menstrual cycle.

b) Exclusion Criteria

The following were the exclusion criteria.

1. Patients with severe gingival inflammation attributable to local irritants such as plaque and calculus or ill-fitting prosthetic appliances.
2. Acute inflammatory conditions of the gingiva such as acute herpetic gingivostomatitis and acute necrotizing ulcerative gingivostomatitis (ANUG)
3. Patients who underwent surgical procedures of the endocrine glands or ovaries
4. Patients on hormone replacement therapy (HRT) for any disease
5. Patients with irregular menstrual history
6. Patients who are pregnant or had any recent history of miscarriage
7. Patients on hormonal contraceptives
8. Patients with systemic contributing factors for gingival inflammation
9. Patients who are mouth breathers
10. Patients who are smokers
11. Patients undergoing orthodontic treatment
12. Patients who are diabetic
13. Uncooperative patients who were not willing to take part in the study

A Proforma was prepared to record the details of the subjects included in the study. The subjects were in the age group between 25 and 60 years.

Prior to carrying out the study, the objectives of the study were explained to all the subjects in a language the subjects could understand and patient's explicit consent was obtained in the Consent Form.

This was followed by a thorough history taking and intra oral clinical examination as outlined in the Proforma. In this study, the standard used for the clinical appearance of desquamative gingivitis included gingival erythema not resulting from plaque, gingival desquamation, other intraoral and sometimes extraoral lesions, and complaints such as burning mouth after eating spicy foods[23, 24]. The clinical criteria also included the presentation of fiery, red, friable gingiva which is painful and desquamates easily and the involvement of buccal aspect of attached gingiva which were not significantly improved by oral hygiene measures alone. 21 Based on these clinical parameters, the free and attached gingiva

of all the patients were examined under good illumination and after drying the surface. The serum estrogen level was estimated in all the 100 patients.

After adopting proper aseptic precautions, 4.0 ml blood was drawn from each of the subjects from the median cubital vein and immediately the sample was sent for the estimation of serum E2 level. Human serum (including serum collected in serum separator tubes) or plasma collected in lithium heparin (including plasma separator tubes) or potassium EDTA collected in glass or plastic may be used in the Architect Estradiol Assay. In the clinical laboratory, the sample thus obtained is inspected for any air bubbles. If any air bubbles are present, they are removed with a disposable applicator stick. The serum specimen is centrifuged after complete clot formation; otherwise, presence of fibrin, red blood cells or other particulate matters in the serum may cause erroneous results. The specimen may be stored for up to 7 days at 2-8˚C before being estimated for serum E2 level. The sample from the middle of the tube is taken for estimation mainly to avoid any particulate matter on the top or bottom of the specimen.

The Architect Estradiol Assay is a delayed one-step immunoassay to determine the presence of estradiol in human serum and plasma using Chemiluminescent Microparticle Immuno Assay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Architect i system manufactured by Abbot Ireland, Diagnostic Division was the laboratory equipment used for the assay.

In the first step, sample, specimen diluent, assay diluent, and anti-estradiol (rabbit, monoclonal) coated paramagnetic microparticles are combined. Estradiol present in the sample binds to the anti-estradiol coated microparticles. After first incubation, estradiol acridinium labeled conjugate is added to the reaction mixture. After a second incubation, and washing, Pre-Trigger and Trigger solutions are then added and the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of estradiol in the sample and the RLUs detected by the Architect optical system. The installed Estradiol assay file on the Architect i system helps to get assay parameter.

The Architect i system is loaded with the reagent kit. The reagent carousel has color coded rings which match the color bands on the reagent bottle labels. The sample is loaded. When the system runs, the sample and the reagents are loaded into the reaction vessel and measures chemiluminescent emission to determine the quantity of estradiol in the sample. The system then automatically calculates and reports the result. The estradiol test result is expressed as pg/mL. The average serum E2 level in normal menstruating females can vary from 21 to 443 pg/mL and less than 20 to 28 pg/mL, in post-menopausal women [10].

IV. Results and Observations

Group A consisted of 50 female patients belonging to the study group having clinically diagnosed chronic desquamative gingivitis and Group B consisted of 50 female subjects who were normal. Group A and Group B subjects belonged to the age group of 25 to 60 years of age. The mean age of the Group A patients was 44.52 ± 10.52. The mean age of the Group B subjects was 36.32 ± 8.32. The lowest age of the Group A patients was 25. The lowest age of the Group B subjects was 25. The highest age of the Group A patients was 60. The highest age of the Group B subjects was 60 (Table 1).

Table 1 : Age of the patients across Group A and Group B samples

AGE (in years)					
Group A			Group B		
Mean ± SD	Lowest	Highest	Mean ± SD	Lowest	Highest
44.52 ± 10.52	25	60	36.32 ± 8.32	25	60

The mean serum estradiol (E2) level of the Group A patients was 18.92 ± 18.05. The mean serum estradiol (E2) level of the Group B subjects was 66.44 ± 67.48. The lowest serum estradiol (E2) level in the Group A patients was 10. The lowest serum estradiol (E2) level in the Group B patients was 10. The highest serum estradiol (E2) level in the group A patients was 92. The highest serum estradiol (E2) level in the Group B subjects was 284 (Table 2).

Table 2 : Serum E2 level across Group A and Group B samples

SERUM E2 (in pg/ml)					
Group A			Group B		
Mean ± SD	Lowest	Highest	Mean ± SD	Lowest	Highest
18.92 ± 18.05	10	92	66.44 ± 67.48	10	284

In univariate analysis (Table 3), among the subject group (Group A), 60 % were showing age more than 40 and in control group (Group B), 32 % were showing age more than 40. The distribution of age is significantly different in subject and control groups.

Table 3 : Comparison of variables across Group A and Group B (univariate analysis)

Variables		Group A		Group B		Odd's ratio	p – Value
		Number	%	Number	%		
Age	≤ 40	20	40.0	34	68.0	3.18	0.005
	> 40	30	60.0	16	32.0		
Serum	≤ 20	40	80.0	10	20.0	16.0	<0.001
	>20	10	20.0	40	80.0		
Menopause	No	32	64.0	47	94.0	8.84	0.001
	Yes	18	36.0	3	6.0		

Table 4: Comparison of variables across Group A and Group B (multivariate analysis)

Variables		Group A		Group B		Odd's ratio	p – Value
		Number	%	Number	%		
Age	≤ 40	20	40.0	34	68.0	1.36	0.618
	> 40	30	60.0	16	32.0		
Serum	≤ 20	40	80.0	10	20.0	13.8	0.000
	>20	10	20.0	40	80.0		
Menopause	No	32	64.0	47	94.0	2.23	0.326
	Yes	18	36.0	3	6.0		

There is a high percentage of low serum E2 level in Group A (80%) compared to Group B (20%) showing significant association ($p < 0.001$). Odd's ratio is 16.0. Compared to Group B there is 16 times more chance of low level of serum E2 in Group A.

In Group A, 36 % had attained menopause. In control group, 6 % had attained menopause. Compared to Group B, there is 8.8 times more chance of menopause in Group A.

Multivariate logistic regression analysis was done with age, estradiol levels and menopausal status as covariates. Among these covariates, only serum E2 level was showing significant independent risk for chronic desquamative gingivitis. Odd's ratio is 13.8 which mean 13.8 times more chance of association of chronic desquamative gingivitis with low serum E2 level.

V. Discussion

Desquamative gingival diseases were descryibed in the late nineteenth century by Tomes and Tomes in 1894, who noticed a singular modification of chronic inflammation of gums, in which, instead of becoming thickened and irregular on the surface, they appeared rather to decrease in size, assuming a very smooth, polished and mottled surface. The patients suffering from this complaint were poor, middle-aged women in whom menstruation was becoming irregular or had altogether ceased.

Early investigators believed gingival lesions that developed in postmenopausal women were primarily the result of a change in their hormonal status. However in the mid-twentieth century, researchers found that chronic desquamative gingivitis was probably a manifestation of several diseases with multiple etiologies. Markopoulos A. K, et al. in 1996[27] stated that 12 % of 414 patients with desquamative gingivitis, approximately 51% were associated with mucoc-utaneous diseases and the rest with idiopathic or hormonal etiology. However, Crispian Scully and Stephen R. Porter in 1997,[2] said that desquamative gingivitis is usually related to mucocutaneous disorders such as mucous membrane pemphigoid and lichen planus, chemical damage and allergic response due to mouth washes, chewing gum, or dental materials and drugs. If there are several different disease entities, the contributions of sex steroid hormones in the initiation and progression of specific desquamative lesions are largely undefined. Circum-stantial clinical data are available to suggest that sex steroid hormones may play a role in some types of desquamative gingival lesions.

Hiyarasu Endo and Terry D. Rees in 2011[28] described the standard used for the clinical appearance of desquamative gingivitis which included gingival erythema not resulting from plaque, gingival desquamation, other intraoral and sometimes extraoral lesions, and complaints such as burning mouth after eating spicy foods. Clinically, the lesion appears as fiery red, glazed, atrophic and eroded-looking, diffuse erythema of marginal and attached gingiva with areas of desquamation and pseudo-membrane formation.[29] Most patients with desquamative gingival lesions are middle-aged and approximately 80% are female.

The correct diagnosis of underlying disease in desquamative gingivitis patients requires careful clinical examination, detailed medical history, biopsy and histopathological examination and the more specialized tests such as direct and indirect immunofluorescence.[30]

A number of studies suggest that oral soft tissues are sensitive to hormonal imbalance. In a study by Daniel, et al.[8] 12 patients belonging to age group from 21 to 67 years (10 women and 2 men) were analyzed and suggested a local depletion of estrogen in

the oral tissues as a major causative agent. R.W. Wardrop, et al. in 1989[30] stated that oral discomfort was found to be significantly higher in peri-menopausal and post-menopausal women who reported improvement with hormone replacement therapy. Eliasson, et al. in 2003[31] in their study stated that HRT can relieve oral discomfort in post-menopausal women. Exogenous estrogens have been used to successfully treat desquamative lesions. This piece of evidence suggests that some lesions are estrogen sensitive and could be due to the low level of serum estrogen.

In normal menstruating females, the level of serum estrogen is 20 - 145 pg/ml during the follicular phase, 112 - 443 pg/ml during mid-cycle phase and 20-241 pg/ml during luteal phase. In post-menopausal females not on HRT, the level is 10 - 28 pg/ml.[10]

Decreased serum estrogen level is associated with many metabolic conditions. Osteopenia, osteoporosis and progression of periodontitis was found associated with low serum E2 level.[32] According to Bonnie J. Deroo, et al.[26] estrogen has wide spread role in human physiology and is implicated in the development and progression of numerous diseases, which include osteoporosis, neurodegenerative diseases, cardiovascular disease, insulin resistance, lupus erythematosus, endometriosis, obesity and various types of cancer such as breast, ovarian, colorectal, prostate and endometrial. In many of these diseases, estrogen mediates its effect through the estrogen receptor (ER), which serves as the basis for many therapeutic interventions.

Physiological and pathological response of the tissue to hormone depends on the reaction between hormone and its special receptors in the tissue because for direct response to hormone, the tissues need to have specific receptors of that hormone. The estrogen receptors are present in the non-target organs such as gingiva. The oral soft tissues are sensitive to changes in serum levels of sex steroid hormones, especially in females.[12] Chebowski, et al.3[32] and Amar, et al.[33] have stated that human gingiva can metabolize estrogen and contains specific high-affinity estrogen receptors. Masaharu Miyagi, et al.[23] hypothesized that sex hormones may affect inflammation through their actions on the function of polymorphonuclear leukocytes (PMNs) and monocytes. In their study, the chemotactic ability of PMNs was reduced by estradiol by binding to the cytoplasmic estrogen receptors. They suggested that the altered PMN chemotaxis associated with gingival inflammation may be due to the effects of sex hormones.

Maryam Seyedmajidi, et al.[34] stated that hormone receptors can be identified using ligand bonding, auto radiography, immunohistochemistry such as reverse transcriptase polymerize chain reaction and in situ hybridization.

Women experience hormonal variations in both physiological and nonphysiological conditions. Female sex hormones (Estrogen) have significant biological actions that can affect other organ systems including gingiva as reported by Salomon Amar, et al. in 1994.[32] Parker, *et. al.*[35] conducted polymerase chain reaction analysis on oestrogen and androgen receptor expression in human gingival and periodontal tissue and found that the gingival inflammation seen during sex hormone imbalance in vivo could be due to secondary effects of estrogen, perhaps on the leucocytic infiltrate present in the inflamed periodontal tissue.

In the current study, Group A and Group B female subjects belonged to the age group from 25 to 60 years of age. The age group was so determined mainly to avoid observer bias and in order to obtain more accurate result. The bias which would have occurred due to the physiological decline in the E2 level following menopause was thus eliminated. Girls usually attain menarche during 13- 16 years of age. There are irregularities of menstruation in some, during the early years. The minimum age selected was 25 years because the serum E2 level was expected to be stabilized in this age group. The mean age of Group A subjects (CDG patients) was 44.52 ± 10.52 years. The mean age of Group B control subjects was 36.32 ± 8.32 years.

In the present study, it was noted that 20 % of control subjects were having a low serum estradiol level, less than 20 pg/ml whereas 80 % were having normal or more than 20 pg/ml. About 32 % of control patients were above 40 years or in the pre-/peri-menopausal age. This could be the reason for the low serum estradiol level in 20 % patients in control group.

From the current study, it was evident that 40% of the subjects of group A were in the age group below 40 years and 60 % of CDG patients were above 40 years whereas in the control group 68 % were below 40 years and 32 % were above 40 years ($p = 0.618$). So it can be inferred that, since patients less than 40 and more than 40 simultaneously presented with CDG, it cannot be stated that age of the women had a direct correlation to the development of CDG.

In the control group, 94 % had not attained menopause and only 6 % had attained menopause. Among the patients who had CDG, it was observed that 64 % had not attained menopause and 36 % had attained menopause ($p = 0.326$). This clearly shows that even patients who had not attained menopause had developed CDG. Hence the variable of menopause and related hormonal fluctuations could not be considered to be statistically significant.

In the current study, the serum E2 level of Group A ranged from 10 - 92 pg/ml with a mean level of 18.92 ± 18.05 and that of Group B ranged from 10 - 284 pg/ml with a mean level of 66.44 ± 67.48. After multivariate logistic regression analysis, it was observed

that 80 % of chronic desquamative gingivitis patients (Group A) were having serum E2 level less than 20. It was only 20 % of the CDG patients who had normal serum E2 level. Whereas in control group, 80 % were with normal serum E2 level and only 20 % had decreased serum E2 level ($p = 0.000$). From this it is clear that there is a significant direct correlation between low level of serum E2 and the development of CDG.

Decrease in serum E2 levels was seen in all menopause patients with CDG. However, it is interesting to note that 69 % of patients with CDG, who had not attained menopause also had a decreased E2 level. This further reinforces the fact that irrespective of age and menopause, decreased E2 levels in CDG patients has a correlation with each other.

CDG in 20% of the subjects with more than 20 pg/ml may be due to idiopathic cause or may be associated with other disorders as it could be the first clinical sign and symptom in many ulcerative and vesiculobullous diseases.

In practice, long-term steroids are the mainstay for the management of CDG. Considering the side-effects of steroids, it could be beneficial to find alternative modalities of management for CDG. Hence, in cases of desquamative gingivitis not responding to steroids or in patients with low serum E2 values, topical estrogen ointments or low dose hormone replacement therapy could be considered under the careful supervision of a physician or gynaecologist.

The aim of the study was to find out if there were any correlation between CDG and serum E2 level and it is clear from the results that in 80 % of patients with CDG, serum estradiol level was low. The findings in this study should be considered as preliminary observations because only a small number of patients with CDG were analyzed. Further randomized control trials are merited to establish the linkage between low serum estradiol (E2) level and CDG. It would also help to assess treatment outcomes with estrogen supplements for patients with CDG.

VI. Conclusion

Desquamative gingivitis is not a disease but a reaction pattern of gingiva which conceals other pathologic diseases. Hormonal imbalance has been suggested as one of the etiology. In the present study, the level of circulating estradiol (E2) was found to be decreased in chronic desquamative gingivitis.Further investigative studies to find out the effect of exogenous estrogen in the management of desquamative gingivitis could be done. In this study, the severity of CDG and serum level of E2 was not compared. This could also be done in future studies.

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Implant Surface Micro-Design

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Abstract- The application of implants for dental and orthopedic surgery has increased rapidly within the past few decades. In craniomaxillofacial surgery, different implant systems have been applied, for example, for dental and bone replacement or osteosynthesis plates and screws. These implants may be made of pure titanium or a titanium alloy, usually titanium-aluminum-vanadium (Ti-6Al-4V). The surface can he turned or Machined or a coating may cover the metal base. The reason for treating the implant surface is to obtain maximum bone-implant contact and bone-implant stability and to shorten the healing time for earlier loading. The crucial aspect of pure titanium implants is the development of titanium oxide on the surface. This oxide and other known coatings for implant material do not have high wear resistance.

This article thus aims to review *Implant Surface Micro-design* its rationale, various surface's physical and chemical properties, different types of implant surface treatments, optimum roughness of oxidized implants and controversies associated with various implant topographies. The recent advances like nanotechnology are also included.

Keywords: implants, implant topography, implant surface micro-design, implant surface treatments.

GJMR-J Classification: NLMC Code: WU 158

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Implant Surface Micro-Design

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Abstract- The application of implants for dental and orthopedic surgery has increased rapidly within the past few decades. In craniomaxillofacial surgery, different implant systems have been applied, for example, for dental and bone replacement or osteosynthesis plates and screws. These implants may be made of pure titanium or a titanium alloy, usually titanium-aluminum-vanadium (Ti-6Al-4V). The surface can he turned or Machined or a coating may cover the metal base. The reason for treating the implant surface is to obtain maximum bone-implant contact and bone-implant stability and to shorten the healing time for earlier loading. The crucial aspect of pure titanium implants is the development of titanium oxide on the surface. This oxide and other known coatings for implant material do not have high wear resistance.

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I. Introduction

The success and predictability of osseointegrated dental implants have forever changed the philosophy and practice of dentistry and, perhaps more than any other specialty, Prosthodontics has changed dramatically. In the late 1950's, Per-Ingvar Branemark, a Swedish professor in anatomy studying blood circulation in bone and marrow, developed through a serendipitous finding in the history of medicine: he predictably achieved an intimate bone-to-implant apposition that offered sufficient strength to cope with load transfer. He called the phenomenon "osseointegration"..

Since that time, millions of patients have been treated worldwide using this technique. The implants used sometimes had different geometries and surface characteristics. A key element in the reaction of hard and soft tissues to an implant involves the implant's surface characteristics, that is, the chemical and physical properties. Quest continued for a material with a surface property which enhances bone apposition at the implant surface in an osteoconductive manner. The quest was for a biocompatible if not bioactive surfaces, achieved through additive or subtractive process.

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Titanium, preferably commercially pure titanium, became the standard for endosseous implants. Actually titanium is a very reactive material that would not become integrated with tissues. However, its instant-aneous surface oxidation creates a passivation layer of titanium oxides, which have ceramic- like properties, making it very compatible with tissues.

II. Rationale for a Dynamic Implant Surface

Oral implant is an alloplastic material or device that is surgically placed in to the oral tissue beneath the mucosal or periosteal layer or within the bone for functional, therapeutic, or esthetic purposes[1]. More needs to be known about the optimal situation of the connection between an artificial material and the tissues-what type of material that gives the best tissue response and what type of surface is preferred by the bone cells or the cells in the soft tissue. If this is known, the response of the bone or soft tissue can be predicted when the implants are installed into the jaws. There is some information and understanding of the effect of design and toxicology of the implants, surgery techniques, effect of movement of the implant during the healing period and biodegradation. Understanding is lacking, however, of the relationship between the events that occur at the implant surface and the effect the implant material has in the tissue and the biocom-patibility of the material[2].

a) The bone-implant interface

Bone tissue is a living organ, which can be described as a natural composite composed of an organic matrix strengthened by an inorganic calcium phosphate (CaP) phase. The extracellular organic matrix (ECM) of bone consists of 90% collagenous proteins and 10% non-collagenous proteins. Regarding the inorganic component, the most abundant mineral phase in human bone is carbonate rich hydroxyapatite (with a carbonate content between 4% and 8%)[3].

When an implant is installed in a jaw, a series of reactions take place on the implant surface. The implant is exposed to a series of different ions, to polysacc-harides, carbohydrates and proteins as well as to such cells as chondroblasts, fibroblasts and osteob-lasts that react with the surface (Figure:1 and 1a)[2,3]. The initial reactions between the tissue constituents and the implant surface govern the further reactions and determine the biological activity of the surface and the further cell responses to the surface. This tissue

response depends on the nature of the surface and its chemical properties, which influences the nature of the subsequent composition of the protein film that adsorbs onto the material [4-7]. this further strongly influences the cell responses on the surfaces.

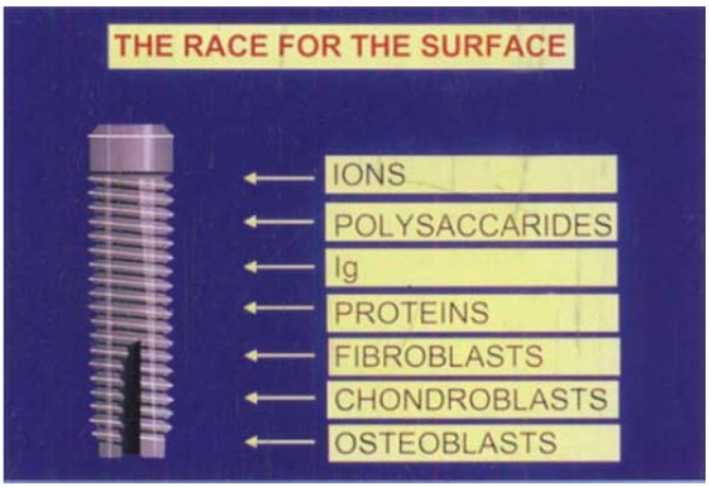

Figure 1 : After implantation, the biomaterial is exposed to a series of different tissue constituents that react with the surface. The type of reaction that occurs probably influences the further cell reactions and finally the tissue-biomaterial connection. Ig: immunoglobulins

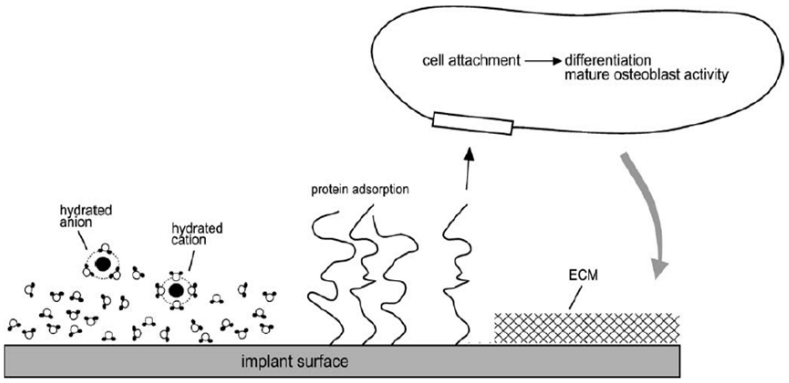

Figure 1(a) : Schematic representation of events consecutively taking place at the titanium surface after implantation into living bone tissue. Water binds to the surface, followed by incorporation of hydrated ions, adsorption and desorption of proteins, eventually leading to cell attachment. After differentiation, mature osteoblasts produce the extracellular matrix (ECM)

b) Osseo-integration versus Osseo-coalescence

The term osseointegration largely refers to the physical integration or mechanical fixation of an implant in bone. The interlocking provides mechanical resistance to forces such as shear experienced in "pull-out" and "torque-out". With purely physical interaction, however, the interface would not be able to withstand even moderate tensile forces. The term osseocoalescence has been proposed to refer specifically to chemical integration of implants in bone tissue. The term applies to surface reactive materials, such as calcium phosphates and bioactive glasses, which undergo reactions that lead to chemical bonding between bone and biomaterial. With these materials, the tissues effectively coalesce with the implant[8].

III. Physical Properties

Several authors have discussed the dimension of the ideal roughness that would provide increased retention and an improved bone response. The roughness can be considered on different levels: macrostructural, microstructural and ultrastructural, and roughness on these different levels probably has different effects on the living tissues. It has been established in the literature based on several studies that, to gain complete growth of bone into a material's irregularities, these need to be at least 100 μm in size. Growth of bone into cavities or pores of this size will give a mechanical interlocking of the material with bone. This was demonstrated by Bobyn et al. in studying cobalt-based alloys with pore sizes of 50- 400 μm[9], Bone

ingrowth was also observed by Clemow et al. when this group studied porous coated Ti&I,V femoral implants with pore sizes ranging from 175 to 235 μm[10].

a) Surface Microstructure

This can vary considerably depending on the surface treatment of the implant. Variation of the surface microstructure has been reported to influence the stress distribution, retention of the implants in bone and cell responses to the implant surface. The implants with rough surfaces have improved bone response, with bone trabeculae growing in a perpendicular direction to the implant surface. An improved retention in bone has also previously been reported after implantation of rough-surfaced implants[2].

Surface roughness on a smaller scale was, however, found to be important for integration of the bone with the implant surface[11]. Although surface roughness on a micrometer scale gives some retention due to bone in growth, *in vitro* cell studies indicate that this property of the surface influences the function of the cells, the matrix deposition and the mineralization[12]. Cells seem to be sensitive to microtopography and appear to be able to use the morphology of the material for orientation and migration[13]. The maturation of the cells also affects the response to the surface roughness, which is in agreement with earlier observations that indicated that chondrocytes are affected differently by local factors such as vitamin D and transforming growth factor p depending on the stages of maturation of the cells[14,15]. Microtopography may therefore be one factor that influences the differentiation of mesenchymal cells into fibroblasts, chondrocytes or osteoblasts. Based on these studies, it can be hypothesized that osteogenesis may be favored by vascular in growth, whereas a limited vascular in growth may induce chondrogenesis. Figures: 2 and 4.

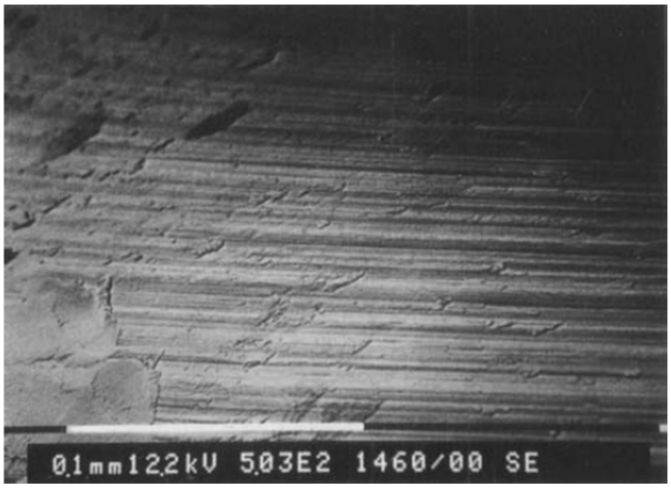

Figure 2 : Scanning electron micrograph with high resolution (x503) of the surface of a machined, threaded implant (Nobel Biocare Mark II)

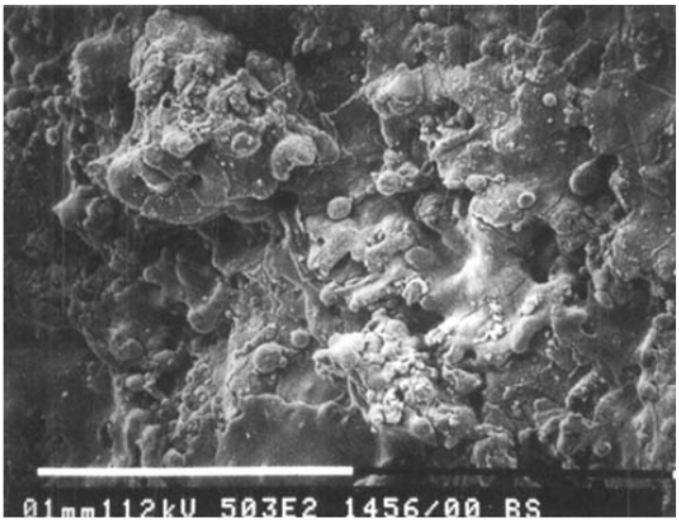

Figure 3 : Scanning electron micrograph with high resolution (x503) of the surface of a titanium plasma-sprayed threaded implant (IT1 Bonefit)

Figure 4 : Scanning electron micrograph with high resolution (X503) of the surface of a titanium dioxide-blasted threaded implant (Astra Tech TiO-blast)

The ideal surface roughness for bone implants on a micrometer scale probably depends on the distribution of cortical or cancellous bone and on the level of loading to the implants.[2] The rugofile bone cells recognizes the surface prepared by the course particle, as a smooth surface, whereas the 25-pm particles creates a rough surface that is identified by the osteo-blasts[2] Figure: 5.

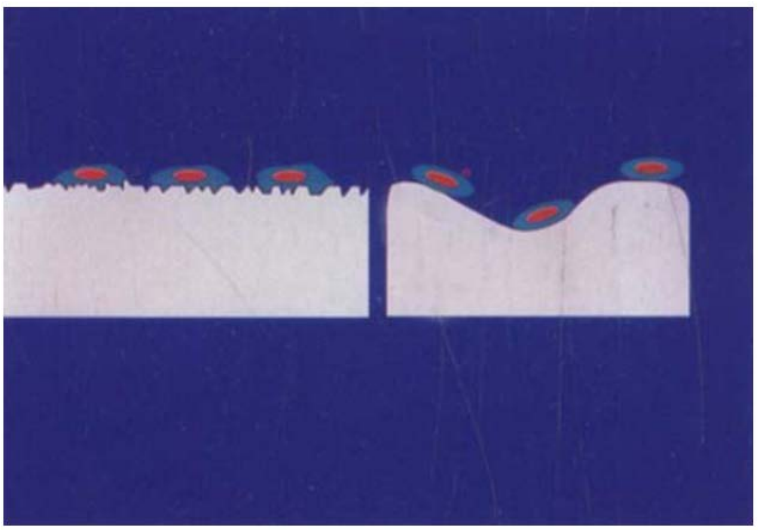

Figure 5 : Bone cells exposed to a medium rough and a very rough surface. The rugofile bone cells may recognize the very rough surface (right) as a smooth surface, whereas the medium rough surface (left) is recognized as a trough rough surface by the osteoblasts

Osteoblasts respond to microarchitectural features of their substrate. On smooth surfaces (tissue culture plastic, tissue culture glass, and titanium), the cells attach and proliferate but they exhibit relatively low expression of differentiation markers in monolayer cultures, even when confluent. When grown on microrough Ti surfaces with an average roughness of 4-7 μm, proliferation is reduced but differentiation is enhanced and in some cases,as it is synergistic with the effects of surface microtopography. In addition, cells on microrough Ti substrates form hydroxyapatite in a manner that is more typical of bone than do cells cultured on smooth surfaces. Osteoblasts also respond to growth factors and cytokines in a surface-dependent manner. On rougher surfaces, the effects of regulatory factors like 1α, $25(OH)_2$ D_3 or 17β-estradiol are enhanced. When osteoblasts are grown on surfaces with chemistries or micro architectures that reduce cell attachment and proliferation, and enhance differe-ntiation, the cells tend to increase production of factors like TGF β1 that promote osteogenesis while decreasing osteoclastic activity. Thus, on microrough Ti surface, osteoblasts create a microenvironment conducive to new bone formation[16]. Figure:6.

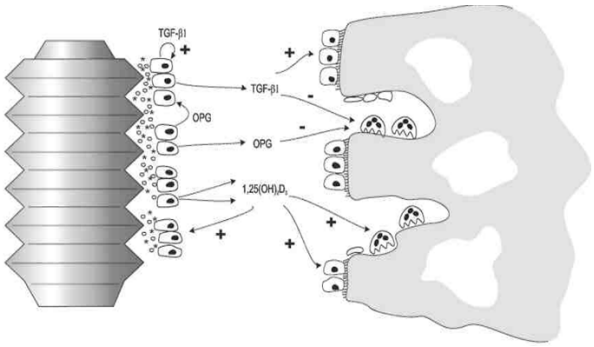

Figure 6 : Schematic diagram showing the effects of rough microtopography on production of paracrine factors by osteoblasts during peri-implant bone formation. Osteoblasts synthesize osteoid on the implant surface as well as on the normal bone surface. Levels of latent TGF-β1 are increased in the extracellular matrix, as well as in the extracellular fluid. Once activated, the growth factor can stimulate osteoblast proliferation, extracellular matrix synthesis and alkaline phosphatase activity (+). At the same time, active TGF-β1 inhibits osteoclastic activity (-). Osteoblasts also produce elevated levels of 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on rough surfaces. 1, $25(OH)_2$ D_3 promotes osteoclast formation due to fusion of monocytes and acts on osteoblasts promoting their differentiation. 1α, $25(OH)_2$ D_3 also stimulates matrix calcification through rapid activation of Ca2+ ion deposition

b) Surface Ultrastructure

Although micro-roughness seems to be an important characteristic for tissue response to biomaterials, there are also observations that indicate a biological response to irregularities on the nanometer level. Larsson et al. studied the biological effect of changing the oxide thickness of titanium implants from an electropolished level, to thick oxide layers formed by anodization. By this treatment the surface changes from an amorphous metal surface with a noncrystalline oxide to a polycrystalline metal surface with a crystalline oxide layer[17].

Analysis of these surfaces at a high resolution level demonstrated that the new surface was heterogeneous with mainly smooth areas of thick oxide but separated with porous regions on a nanometer level. This observation of an increased roughness after anodization of titanium was in line with earlier transmission electron microscopic studies demonstrating increased pore sizes with increased oxide thickness[18].

Implants with this thick, heterogeneous oxide seemed to have a slightly improved response in bone, particularly in the first weeks after implantation.

c) Smooth versus Rough Surfaces

Surface quality of an oral implant can be subdivided in to mechanical, topographic, and physic-chemical properties[19]. Surface topography is characteristic of the preparation process. Variations in the roughness and porosity can be categorized in function of the surfacing process. The current state of information regarding implant surface topography has provided clinicians with confusing options. Machined implants are not smooth, and not all rough implant surfaces are equivalent. Surfaces often are identified by the method of manufacture and not the resultant surface.

Rough surfaces can be divided into three levels depending on the scale of the features: macro-, micro- and nano-sized topologies. The macro level is defined for topographical features as being in the range of millimeters to tens of microns[20]. This scale is directly related to implant geometry, with threaded screw and macro porous surface treatments giving surface roughness of more than 10μm. Numerous reports have shown that both the early fixation and long-term mechanical stability of the prosthesis can be improved by a high roughness profile compared to smooth surfaces[21].

The high roughness resulted in mechanical interlocking between the implant surface and bone on growth. However, a major risk with high surface roughness may be an increase in peri-implantitis as well as an increase in ionic leakage. A moderate roughness of 1–2μm may limit these two parameters[22]. The microtopographic profile of dental implants is defined for surface roughness as being in the range of 1–10μm.

IV. Chemical Properties

a) The surface chemistry of the implants

The chemical properties of the biomaterial surface play an important role for the tissue responses elicited by the material. This is at least one main reason why the tissues responds differently to different materials.[2] A material with a surface that is accepted by the tissue seems to exhibit improved integration with bone, either due to passive growth, leading to a tight connection between implants and bone, or by stimulation that probably leads to a bone-implant bonding. This is probably the case with the two main materials used in dental implants, hydroxyapatite and

titanium.[2] The calcified parts of the bone consists of hydroxyapatite (or rather carbonated apatite), and introducing this substance as an implant material often gives favorable responses in the bone.[23]

The biological effects of modifying the biomaterial surface have also been elaborated[24-25]. In an attempt to study the effect of the oxide layer of titanium on calcium-phosphate precipitation, titanium-dioxide (TiO2) and powder of oxidized and nonoxidized titanium were introduced into an in vitro nucleation test system[24]. In this system they found that titanium powder enhances calcium phosphate nucleation only after prolonged pre-incubation in an aqueous buffer, or after autoclaving. These treatments enhance the growth of the oxide layer. This observation indicated that the oxide content, or structure, is required for titanium to act as a nucleation substrate. Even more effective nucleation was observed when pure TiO2 was used as a nucleation substrate. The nucleation capacity and formation of calcium phosphate precipitates is related to the biocompatibility of titanium, and enhanced nucleation capacity may indicate improved biocompatibility.[2]

The biological activity of the TiO_2 probably also influences the protein adsorption to titanium. In an *in vitro* study, serum proteins seemed to adsorb to titanium dioxide by the same mechanisms as to hydroxyapatite through calcium binding[24]. The surface characteristics of TiO2 probably change from an anionic to a cationic state by the adsorption of calcium to the surface. This will subsequently increase its ability to adsorb acidic macromolecules, such as albumin, a property demonstrated for hydroxyapatite[26-27].

Fluoride ions have documented activity in bone. This element is known to form fluoridated hydroxyapatite or fluorapatite with improved crystallinity and better resistance to dissolution than hydroxyapatite[28]. Fluoride also enhances the incorporation of newly formed collagen into the bone matrix and increases the rate of seeding of apatite crystals as well as increasing trabecular bone density and stimulating osteoprogenitor cells number *in vitro* [29,30]. Figure-7 and 8.

Figure 7 : Scanning electron micrograph of a fluoride-modified implant after the push-out procedure. The implant is partly (right side) covered by bone that is firmly fixed to the implant surface, which indicates bonding between the titanium implant and bone

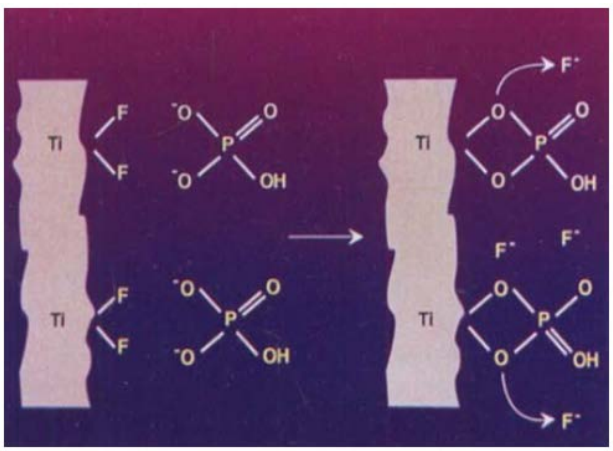

Figure 8 : A possible mechanism between the fluoride-modified titanium and bone. Oxygen in phosphate may replace the fluoride and bind to titanium to create a covalently binding between bone and titanium. The fluoride ions which are released by this process may thus catalyze the new bone formation in the surrounding tissue

V. Diferent Types of Implant Surface Treatments

The desired implant surface can be achieved by addition of material over the surface, removal of material from the surface or modification of the surface material. Some of the examples are:

I. Addition of material - Titanium plasma spray (TPS, TiO2); coating with hydroxyapatite (HA).
II. Removal of material - Particle jets and/or acid etching
III. Modification of material - The implant surface can be modified without either adding or removing material. (Electron beam, thermal treatment, laser treatment, and ion implantation)

a) Addition of Material (Additive Methods)

Chemical substances are successfully added over the surface. Some of the materials used for this purpose include:

1. ydroxyapatite. 2.Titaniumoxide. 3. Titanium nitrite.	4. Zirconium. 5. Fluoride. 6.Nano structured Al.	7.Tantalum chloride. 8. Magnesium. 9.Biologic substances.

Apart from the above mentioned chemical substances, the following biologic materials can also be added over the surface to obtain the desired surface properties:

A. RhBMP-2. B. Growth factors. C. Type 1 collagen.	D. RGD peptides. E. Human mesenchyme. F. Fibronectin.	G. Vitronectin. H. Laminin. I. Human albumin. J. Chitosan.

b) Removal of Material (Subtractive Methods)

This technique involves creation of surface roughness by various methods like:

1. Sand Blasting. 2. Machining. 3. Micro machining.	4. Acid Etching. 5. Dual Acid Etching.	6. Laser Etching. 7. Micro arc oxidation.

c) Modification of Material

1. Surface Wetting. 2. Plasma cleaning.	3. Electron beam. 4. Thermal treatment.	5. Ion implantation.

VI. Additive Methods of Surface Treatment

a) Hydroxyapatite coating

Hydroxyapatite is a calcium phosphate ceramic that is an osteophilic, osteoconductive, bioactive coating, which is totally biocompatible and becomes an integral part of living bone tissue. Hydroxyapatites and tricalcium phosphates have an excellent grade of acceptance, and these materials may be more rapidly incorporated in bone than commercially pure titanium. Hydroxyapatite coating over titanium has enjoyed a rapid growth because of its inherent biomaterial properties that some consider an advantage over uncoated surgical titanium. Hydroxyapatite (HA) coating has become popular for load bearing dental implants because it elicits a faster bony adaptation, absence of fibrous tissue seams, firmer implant bone attachment, reduced healing time, increased tolerance of surgical inaccuracies, and inhibition of ion release[31].

The first clinical use of hydroxyapatite (HA) as a coating on dental implants began in February 1984, with the results showing many benefits over the no coated implants31. Later, many researchers conducted studies and obtained promising results.

Contemporary plasma-sprayed hydroxyapatite (HA) coatings with high crystalline content are much more resistant to in vivo degradation than HA coatings of a decade ago but reportedly exhibit reduced wettability, which could potentially negatively affect tissue adhesion and long-term clinical outcome.

Bone morphogenetic proteins (BMP s) play a crucial role in cell ingrowth and differentiation in a variety of cell types, including osteoblasts[32]. Because of their beneficial effects, BMP s have been used to accelerate healing after implant placement. Apatite is considered a suitable carrier of BMP-2[33] and the incorporation of BMP-2 into the apatite layer of a titanium implant may enhance its osteoinductive properties.

i. *Methods of HA Coating*

Conventional plasma spraying, flame spraying, and chemical techniques have all been investigated as techniques for producing a thinner HA coating on a metal substrate. The bond formed between HA coatings and the metallic substrate by the spraying method, formed primarily through mechanical interlocking, is not strong enough. Additionally, the spraying method is unsatisfactory for applying a thinner, uniform HA coating on implants because of their complicated shapes. On the other hand, electrochemical methods, electrop-0horetic techniques in particular, seem attractive for forming HA coatings on titanium implants with compli-cated shapes. However, the bond between the coating and the metal substrate is significantly weak. Magnetron sputter coating and Ion Beam sputtering techniques for

coating HA on implant surfaces have been tried with varying rates of success. Hydroxyapatite can be coated by plasma spraying. In this technique, powdered crystalline hydroxyapatite is introduced and melted by the hot, high velocity region of a plasma gun and propelled onto the metal implant as a partially melted ceramic.

b) Plasma Sprayed Titanium

Hahn and Palich (1970) first developed titanium surfaces by plasma spray techniques and reported an enhanced bone ingrowth in those implants. The plasma sprayed titanium surfaces exhibit a porous surface with macro irregularities[34].

i. *Macro-irregularities*

Macro-irregularities in an implant include macroscopic threads, fenestrations, pores, grooves, steps, threads, or other surface irregularities that are visible. The idea is to create mechanical interlocking between implant and bone at the macro level.

ii. *Method of Plasma spraying*

Powdered Titanium is melted at a temperature of 15,000 degrees and is sprayed on to the surfaces of the implant at a very high velocity of 600 m/sec through argon plasma associated with a nozzle. The diameters of the sprayed particles are around .04 to .05mm thickness. When observed microscopically the coatings show round or irregular pores that are interconnected with each other. The surface of the implants where they condense and fuse together, forming a film about 30 μm thick. The thickness must reach 40–50 μm to be uniform. The resulting TPS coating has an average roughness of around 7 μm, which increases the surface area of the implant.

c) Anodic Spark Deposition

Anodic spark deposition techniques have been effectively applied to achieve a microporous morphology on metals. Recently, a new electrochemical process has been developed to improve further the mineralization potential, mechanical stability, and corrosion resistance of the ceramic coating obtained with anodic spark deposition. Electrochemically treated titanium showed promising results and was able to introduce substantial improvements in achieving fast and stable osseointegration of implants in osteopenic sheep bone[35].

d) Biologic Coatings

Puleo and Nanci (1999) emphasized the importance of biochemical methods of surface modification as an alternative or adjunct to morphologic approaches. Biochemical methods are aimed at control of the tissue-implant interface by the immobilization and/or delivery of proteins, enzymes, or peptides for the purpose of inducing specific cell and tissue responses. They rely on current understanding of the biology and biochemistry of cellular function and differentiation and on suitable surface modification techniques[36].

e) Bio- molecules

i. *Laminins*[37]

Laminins are major proteins in the basal lamina, a protein network foundation for most cells and organs. They are an important and biologically active part of the basal lamina, influencing cell differentiation, migration, adhesion as well as phenotype and survival.

ii. *Fibronectin*[37]

Fibronectin is a high-molecular weight (~440 kDa) extracellular matrix glycoprotein that binds to membrane-spanning receptor proteins called integrins.

In addition to integrins, fibronectin also binds extracellular matrix components such as collagen, fibrin and heparan sulfate proteoglycans.

It is involved in cell adhesion, growth, migration and differentiation. Cellular fibronectin is assembled into the extracellular matrix, an insoluble network that separates and supports the organs and tissues of an organism.

iii. *Vitronectin*

Vitronectin is an abundant glycoprotein found in serum the extracellular matrix and promotes cell adhesion and spreading.

Vitronectin serves to regulate proteolysis initiated by plasminogen activation. Additionally Vitronectin is a component of platelets and is thus involved in hemostasis. Vitronectin contains an RGD sequence which is a binding site for membrane bound integrins, e.g. the Vitronectin receptor, which serve to anchor cells to the extra cellular matrix.

iv. *RhBMP-2*

BMP's are Bone morphogenetic proteins. They are members of —growth and differentiation protein family. They are homodimeric, glycosylated proteins that are highly conserved across species. They are found to be osteoinductive in animals and humans.

They are supposed to promote bone induction by increasing Chemotaxis and increasing the proliferation and differentiation of bone forming cells from undifferentiated mesenchymal cells.

They induce the formation of both trabecular and woven bone. The formed bone remodels based on the demand at the particular site. The delivery of BMPs is aimed at local administration, which is in favor for coating the implant surfaces.

v. *Bio molecules and Implants*

The proportions of these biologic molecules and the presence of other lesser-known components seem to vary with the anatomic location and specific function of the individual basement membrane. Ultra structural data provided by Swope and James (1981) indicate that hemidesmosomes formed on Vitallium

implants in monkeys after 2 days and became well established after 3 days[38].

However, more recently published data dispute these findings, indicating that hemidesmosomal contacts were found only on apatite and polystyrene substrates.

vi. *Amino acid sequence RGD*

In a goat femur wound chamber model, Bernhardt et al. (2005) compared bone-to-implant contact on uncoated titanium implant surfaces with RGD peptide-coated surfaces. After 5 and 12 weeks of healing, no significant effect of RGD coating on the mean bone-to-implant contact percentages was observed[39]. These results contradict the findings of Schliephake et al. (2005b) and Rammelt et al. (2006)[40-41].

Schliephake et al. (2005b) compared, in the mandible of dogs, machined titanium implant surfaces (Ti) with RGD-coated implant surfaces. RGD coatings were achieved either with low RGD concentrations (100 m mol/ml) (RGD low') or with high RGD concentrations (1000 m mol/ml) (RGD high). After 1 month of healing, bone-to-implant contact was significantly higher for RGD high compared with Ti. After 3 months of healing, bone-to-implant contact was significantly higher for RGD high and for RGD low compared with Ti[40].

vii. *Collagen and collagen mimetic peptides*

The in vivo osteoconductive potential of type I collagen, type III collagen and collagen mimetic peptide sequences as coating for titanium implants was investigated in the publications of Rammelt et al. (2004,2006,2007), Bernhardt et al.(2005), Schliephake et al.(2005a,2005b) and Reyes et al.(2007)[39-42].

In the proximal tibial metaphysis of rats, Reyes et al. (2007) compared the mechanical anchorage as well as bone-to-implant contact of machined c.p.titanium implant surfaces (Ti)with either bovine type I collagen (Col-I) or glycine–phenyl alanine–hydroxy proline–glycine–glutamate–arginine (GFOGER; a collagen mimetic peptide sequence)-coated implant surfaces. After 4 weeks of healing, the mean pull-out forces were around 35N for GFOGER, 20N for ColI and 35N for Ti. GFOGER was statistically higher compared with ColI or Ti, but the values for Col I were not statistically higher compared with Ti. The authors concluded that both coatings (GFOGER and ColI) enhanced bone repair and implant integration.

viii. *Collagen composite coating with CaP*

In the mandible of dogs, Schliephake et al. (2003) compared bone-to-implant contact between titanium alloy implants with a polished surface (Ti), collagen-coated (Col), mineralized (hydroxyapatite) collagen-coated (Col/HA), sequentially hydroxyapatite-collagen-coated (Col/sew HA) and hydroxyapatite-coated titanium surfaces (HA). Animals were sacrificed after 1 and 3 months of healing. No significant differences in the mean bone-to-implant contact between the various implant surfaces were observed in cortical as well as in cancellous bone after 1 and 3 months of implantation[43].

ix. *Growth factor coatings*

Growth factors are signaling proteins that promote replication, differentiation, protein synthesis and /or migration of appropriate cell types. In case of endosseous titanium implants, an enhanced proliferation and differentiation of undifferentiated mesenchymal cells osteoprogenitor cells and preosteoblasts into osteoblasts may enhance bone healing (Chappard et al.1999)[44].

Therefore, the rational to coat titanium implants with locally acting growth factors is the assumption that the release of these growth factors might improve the remodeling process at the bone–implant interface, leading to enhanced bone response (De Jonge et al. 2008)[45].

x. *Bone Morphogenic Proteins*

A particular class of growth factors, BMPs, has shown considerable potential to stimulate bone formation both in extra skeletal sites (Yamazaki et al. 1996; Yoshida et al. 1998) and in defect models in different species (Zellin & Linde 1997; Teixeira and Urist 1998)46-48. BMPs originate from the TGF-b family and include at least 18 different proteins (Reddi 1995)49. As BMP-2 possesses high osteoinductive potential (Laub et al. 2001), it was considered to be an interesting candidate growth factor to coat titanium implants.

While BMP-2 is used more commonly, BMP-4 is also considered as a candidate growth factor that might improve the remodeling process at the bone–implant interface (Stadlinger et al. 2008)50. Besides promoting bone formation BMPs stimulates recruitment, proliferation, and differentiation of osteoclasts as well (Chen et al.2004)[51].

xi. *Non-BMP growth factors*

Besides BMPs, other growth factors loaded onto titanium implant surfaces were tested in animals as potential agents to enhance osseointegration (De Jonge et al.2008)[45].

Examples are:

1. Growth hormone (GH) (Blom et al.1998)52.
2. Platelet-derived growth factor (PDGF), combined with insulin-like growth factor-1 (IGF-1) (Stefani et al. 2000)53.
3. Platelet rich growth factors (PRGFs)(Fuerst et al. 2003)54 (Eduardo A Anitua 2006)55
4. TGF-b2 (De Ranieri et al.2005)56.
5. Fibroblast growth factor-fibronectin fusion protein (FGF-FN) (Park et al. 2006)57.

xii. *Bone-like coatings*

A method to self-assemble and mineralize collagen gel and to precoat a bone-like layer of

mineralized collagen immobilized on titanium implant surfaces has been demonstrated. The mineralized layer was found to promote cellular activity, indicating potential for more efficient bone remodeling at the implanttissue interface. This may promote and/or accelerate osseointegration[58].

VII. Removal of Material (Subtractive Methods)

Implant Surfaces can be roughened by various material removing techniques. Of which the most common methods are:

1. Sandblasting.
2. Acid etching.
3. Machining.

a) Machining

The machining of Cp titanium imparts a surface roughness that is distinct from smooth or polished surfaces. The machining method is an important determinant of the resulting surface. Different surfaces are imparted by machining or subsequent modification. Electro polishing of machined components can further reduce variations measured at the surface, but such surfaces are not well osseointegrated. Creating topographic variation from the mean surface plane can be achieved by abrasion (TiO2 blasting or soluble/resorbable blasting materials [S/RBM]), blasting, blasting and etching (alumina oxide and H2SO4/HCl), anodizing, cold working (dimpling), and different chemical etching methods (H2SO4/HCl)59-61. Bone to implant contact is one of the important factors for osseointegration. Bone to implant contact is higher for osteotite surfaces when compared to machined surfaces[62] .

b) Grit Blasting

Another approach for roughening the titanium surface consists in blasting the implants with hard ceramic particles. The ceramic particles are projected through a nozzle at high velocity by means of compressed air. Depending on the size of the ceramic particles, different surface roughnesses can be produced on titanium implants. The blasting material should be chemically stable, biocompatible and should not hamper the osseointegration of the titanium implants. Various ceramic particles have been used, such as alumina, titanium oxide and calcium phosphate particles. Alumina (Al2O3) is frequently used as a blasting material and produces surface roughness varying with the granulometry of the blasting media. However, the blasting material is often embedded into the implant surface and residue remains even after ultrasonic cleaning, acid passivation and sterilization. Alumina is insoluble in acid and is thus hard to remove from the titanium surface. In some cases, these particles have been released into the surrounding tissues and have interfered with the osseointegration of the implants. Moreover, this chemical heterogeneity of the implant surface may decrease the excellent corrosion resistance of titanium in a physiological environment[63].

c) Acid-etching

Etching with strong acids such as HCl, H2SO4, HNO3 and HF is another method for roughening titanium dental implants.

Acid etching produces micro pits on titanium surfaces with sizes ranging from 0.5 to 2nm in diameter.[64] Acid- Immersion of titanium implants for several minutes in a mixture of concentrated HCl and H2SO4 heated above100 ◦C (dual acid-etching) is employed to produce a micro rough surface. This type of surface promotes rapid osseointegration while maintaining long-term success over 3 years [65.]
Enhanced bony anchorage was noted to dual acid-etched implants as compared to machined implants[66].

Acid-etched implants showed significantly higher mineral apposition rates compared to acid-etched, phosphate coated implants[67].

d) Sand Blasted and Acid etched (SLA) surface

Among the various techniques to produce a micro rough titanium surface, the combination of sand blasting and acid etching can be used. These surfaces showed enhanced bone apposition in histomorph-ometric studies, and higher torque values in biomec-hanical testing. Based on these experimental studies, clinical studies were initiated to load SLA implants after a reduced healing period of only 6 weeks. The clinical examination up to 3 years demonstrated favorable results, with success rates around 99%[68.]

e) Chemically Modified SLA Surface: SLActive

SLActive is based on the scientifically proven SLAR topography (M. de Wild 2004.). In addition, it has a fundamentally improved surface chemistry. The chemically active, hydrophilic SLActive surface promotes the initial healing reaction, allowing for direct cell interaction at the initial stage of the osseointegration process. Bone formation is immediately initiated resulting in earlier secondary stability and reducing the critical dip.

D.Buser et al studied the modified SLA surface produced by rinsing under N2 protection and storing in an isotonic NaCl solution. They demonstrated that the modSLA surface promoted enhanced bone apposition during early stages of bone regeneration[69].

Michael M. Bornstein et al showed that Dental implants with a mod SLA surface (SLActive) demonstrated statistically significant differences for probing depths and clinical attachment level values compared to the historic control group, with the mod SLA surface implants having overall lower probing depths and clinical attachment level scores[70]. Figure-9.

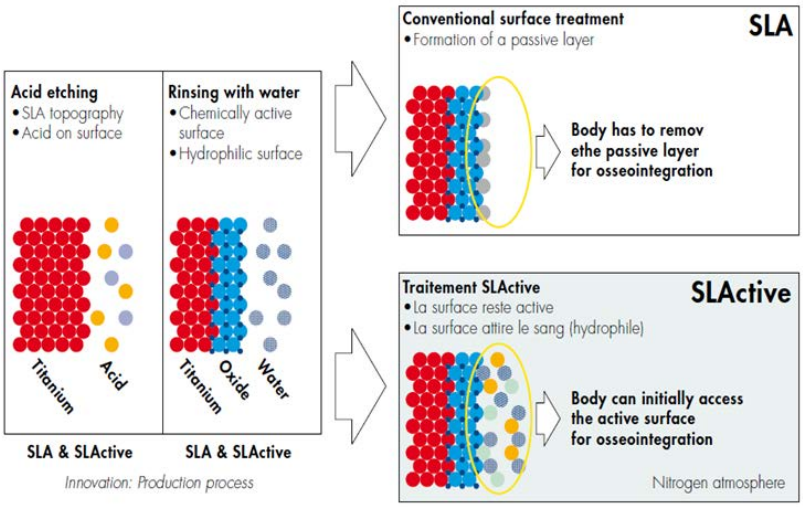

Figure 9 : SLA and SLActive. (F. Rupp, L. Scheideler, N. Olshanska, M. de Wild, M. Wieland, J. Geis-Gerstorfer J. Biomed. Mater. Res. A. 2006;76(2):323–334)

VIII. Modification of Material

a) Ion Implantation

CO ion implantation is a new surface treatment designed to improve implant bone integration by modifying the chemical structure of the implant surface at the atomic level without adding or removing material. This is a high vacuum physical technique (<10-4Pa) in which the surface of a material is bombarded with previously selected and accelerated ions that become integrated or implanted within the outer atomic layers of the surface, thereby modifying the physicochemical properties. A study showed improved percentage BIC values for implants with ion-implanted surfaces in comparison to Diamond like Carbon coating and machined controls. Furthermore, bone integration appeared to be accelerated in the ion implantation group[71].

b) Optimum Roughness

The topography of rough surfaces is characterized by different surface roughness parameters (Ra, Rq, Rt, Rsk, Rku, Δq, or λq, in 3D or 3D mode). Hansson described that an average surface roughness Ra (filtering 50x50 μm) of about 1.5 μm gave the strongest fixation for a bone-metal interface. If the implants are smoother or rougher than this, the anchorage between bone and implant decreases.

A typical measure of implant surface roughness is the Ra value: the arithmetic mean value of the surface departures from the mean plane. Unfortunately, surfaces may have very different morphologies and still share a common Ra value. It is clear that height descriptors alone do not adequately describe surface roughness. More recently, the average peak spacing (Sa) has been associated with implant behavior. There is enough evidence for the positive relationship between surface roughness and increased bone to implant contact (BIC)[72].

c) Optimal Surface Properties

Surface properties of implants directly influence bone responses. Thus, irrespective of the surface modification technology used, detailed surface characterization of an implant is important. Based on the bone response in the present study, which was expressed as a function of quantitative changes in the surface oxide properties, the following appear to be the optimum surface properties of oxidized implants:

- The optimal oxide thickness of a porous surface structure appeared to be in the range of 1,000 to 5,000 nm.
- An optimum porosity of open pores is in the range of 19% to 30%, (i.e.) approximately 24%; with a pore size of 2.0 μm.
- Surface roughness values of 0.7 to 1.0 μm for Sa, 0.9 to 1.4 μm for Sq, and 27% to 46% for Sdr seemed to be optimum.
- TiO2 in a crystalline phase seemed to be optimal[73].

d) Controversies With Respect To Implant Topographies

Machined titanium surfaces have been reported to favor fibroblastic growth, migration, and spread, and therefore were considered favorable for formation of peri-implant soft tissue. On the other hand, because of the increased proliferative activity of fibroblasts on machined surfaces, fibrous capsules or connective tissue overgrowth can form, compromising local blood supply and leading to failure of the implant to integrate with the soft tissue. To overcome this problem, rough titanium surfaces have been suggested in several studies.

Rough titanium surfaces have been reported to improve attachment and decrease growth and spread of fibroblasts. However, a diminished growth of fibroblasts on rough titanium surfaces can result in the formation of a thin connective tissue that will not be capable of supporting surrounding tissue structures. In addition, rough implant surfaces have been reported to be especially prone to peri-implant infection and seem also to attract inflammatory cells.

Another suggested titanium surface comprises grooved topography, which has been demonstrated to favor the orientation and alignment of fibroblasts and claimed in several studies to be appropriate for the establishment of an organized connective tissue structure around the implant. However, the exact topographic configurations of grooved titanium surfaces that are appropriate for the in vivo establishment of long-term stable and overall optimal peri-implant soft tissue conditions are still largely unknown.

There is a lack of knowledge about the ideal implant surface characteristics that lead to the establishment of optimal connective tissue and attachment around titanium implants. The acid-etching and blasting methods generally do not change the main compositional surface elements of the titanium, which consist mainly of titanium and oxygen, but rather the surface morphology/topography and consequently surface roughness, two action mechanisms of osseointegration of oxidized implants have been proposed:

1) mechanical interlocking through bone growth in pores and
2) biochemical bonding[74-75].

e) Surface roughness at the nano scale level

The chemistry and roughness of implant surfaces play a major role in the biological events that follow implantation. Nevertheless, surfaces are often developed using an empirical approach with in vitro and in vivo tests. Most of the surfaces currently available have random topography with a wide range of thicknesses, from nanometers to millimeters[76].

The exact biological role of these features is unknown because of the absence of standardized surfaces with repetitive topography at the nano-sized level (e.g. pits with fixed diameters and depth, lanes with controlled profiles). Such controlled or standardized surfaces might help to understand the interactions between specific proteins and cells. These standardized surfaces might also promote early bone apposition on the implants.

Only a few studies have reported modifications to the roughness as well as the chemistry at the nanometer scale in a reproducible manner. Most of these attempts have used processing methods from the electronic industry such lithography and surface laser-pitting.

These nanometer structures may also give the cells positive guidance by means of the selective attachment of osteoblasts to the implant surface. This selective attachment process might result in the improvement of initial healing around dental implants22.

f) Re-Osseo integration

Persson et al (2001) evaluated reosseointegration of SLA (Sandblasted and acid etched) and turned implants in dogs. They found that reosseointegration was substantial for implants with SLA surfaces but only minimal for exposed smooth (turned) surfaces. Reosseointegration (BIC) at SLA surfaces averaged 84% compared to 22% at turned implant surfaces[77].

IX. Recent Innovations and Future Directions

a) Nanotechnology

Nanotechnology is the engineering of functional systems at the molecular scale. Materials reduced to the nanoscale can show different properties compared to what they exhibit on a macro scale, enabling unique applications. For instance, opaque substances become transparent (copper); stable materials turn combustible (aluminum); insoluble materials become soluble (gold). A material such as gold, which is chemically inert at normal scales, can serve as a potent chemical catalyst at nanoscale. Much of the fascination with nanotechnology stems from these quantum and surface phenomena that matter exhibits at the nanoscale.

Nanotechnology involves materials that have a nano-sized topography or are composed of nano-sized materials. These materials have a size range between 1 and 100 nm (109m) Nanotechnology often involves one-dimensional concepts (nano-dots and nano wires) or the self-assembly of more complex structures (nanotubes). Materials are also classified according to their form and structure as nanostructures, nanocrystals, nano coatings.

b) Methods of Creating Nano-topography

Nanotechnology requires novel ways of manipulating matter in the atomic scale. Several approaches are currently prevalent in the experimental application to endosseous implants.

1. One approach involves the physical method of compaction of nano-particles of TiO2 vs. micron-level particles to yield surfaces with nano scale grain boundaries78. An advantage of this method is that it conserves the chemistry of the surface among different topographies.
2. Second is the process of molecular self-assembly. Self-assembled monolayers (SAMs) are formed by the spontaneous chemisorptions and vertical close-packed positioning of molecules onto some specific substrata, exposing only the end-chain group(s) at the interface . The

exposed functional end group could be an osteo inductive or cell adhesive molecule. An example of this is the use of cell adhesive peptide domains (RGD domains) appended to SAMs composed of poly ethylene glycol (PEG) and applied to the titanium implant surfaces.

3. A third method is the chemical treatment of different surfaces to expose reactive groups on the material surface and create nano-scale topography. This is popular among current dental implant investigators. NaOH treatment catalyzes the production of titanium nanostructures outward from the titanium surface79.
4. The deposition of nanoparticles on to the titanium surface represents a fourth approach to imparting nanofeatures to a titanium dental implant80 Sol–gel transformation techniques achieve deposition of nano meter-scale calcium phosphate accretions to the implant surface81-82. Alumina, Titania, zirconia and other materials can also be applied83. Owing to their resultant atomic-scale interactions, the accretions display strong physical interactions.

a. In a modified approach, Nishimura and colleagues [2007] demonstrated a directed approach to assembly of CaPO4 nano features on dual acid-etched cp Titanium implant surfaces. The deposition of discrete 20–40nm nano-particles on an acid-etched titanium surface led to increased mechanical interlocking with bone and the early healing of bone at the endosseous implant surface in a rat model. One of the main concerns related to coating the implant surface is the risk of coating detachment and toxicity of related debris[84].

5. A fifth approach to creating nano scale topography on Titanium is the use of optical methods (typically lithography) reliant on wave length specific dimensions to achieve the appropriate nano scale modification. These approaches are labor intensive methods that require considerable development prior to clinical translation. The present use of lasers to promote micron-level groove on an implant surface can produce micron-level, not nano scale, modification of the implant surface . Another method of depositing nano scale material on to the implant surface involves ion beam deposition (e.g. hydroxyapatite)[85].

X. Conclusion

Implant surface characteristics are widely recognized as being of fundamental importance in achieving long-term implant success. As such, extensive research has been performed in order to determine the surface texture necessary to attain an optimal bone-implant biomechanical interlock. Four interrelated properties of an implant surface affect osteogenic activity: chemical composition, surface energy, surface roughness, and surface morphology.Osseointegration and its underlying mechanisms of cell attachment, migration, proliferation, and differentiation are sensitive to one or more of these properties. Methods of enhancing the implant surface include alteration of the microstructure and modification of its physiochemical parameters, including surface free energy and wettability.

The surface qualities are of utmost importance in establishing of a reaction between the implant and the tissues. This concerns the surface structure as well as its chemical and biological properties. Much attention has been focused on the importance of the macrostructure of the implants for establishing retention in the bone. More attention will probably be focused in the future on the biological effects of the surface structure on the microstructural and ultrastructural levels as well as on the surface chemistry of the implants. Progress in these fields based on knowledge of the biological effects may provide implants with improved tissue response and clinical performance in the future.

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Effect of Probe-Tip Placement on Impedance Audiometry

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Abstract- *Introduction:* Impedance audiometry measures intactness of middle ear system and acoustic arc. It has become an essential tool in audiological practice, any variability leads to erroneous diagnosis. The most likely error to occur is by different probe-tip placements in the ear canal. The present study was conducted to verify this hypothesis.

Subjects, Material and Methods: The study was conducted on twenty normal hearing subjects (40 ears) with age range of 17 to 28 years. It included measurements of compliance, ear canal volume, middle ear pressure and acoustic reflexes. These parameters were studied for two probe-tip positions (i) ≤1mm; and (ii) 2mm inside the ear canal.

Results: Significant differences were observed between the two probe-tip positions for ear canal volume. During acoustic reflex for 2000 Hz probe-tone, the change in compliance was significantly affected.

Keywords: *probe-tip, placement, tympanometry, acou-stic reflex.*

GJMR-J Classification: *NLMC Code: WZ 112.5.O8*

Strictly as per the compliance and regulations of:

Effect of Probe-Tip Placement on Impedance Audiometry

Sunita Gudwani [ρ], Sanjay Kumar Munjal [α] & Naresh K. Panda [σ]

Abstract- *Introduction:* Impedance audiometry measures intactness of middle ear system and acoustic arc. It has become an essential tool in audiological practice, any variability leads to erroneous diagnosis. The most likely error to occur is by different probe-tip placements in the ear canal. The present study was conducted to verify this hypothesis.

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Results: Significant differences were observed between the two probe-tip positions for ear canal volume. During acoustic reflex for 2000 Hz probe-tone, the change in compliance was significantly affected.

Conclusions: The results can be explained by ear canal resonance principles. Thus, the study verifies that the placement of probe-tip affects the measurements of tympanometry and acoustic reflex testing.

Aim and Objectives: To study the changes of tympanometric and acoustic reflex test measures with different placements of probe-tip.

Keywords: probe-tip, placement, tympanometry, acoustic reflex.

I. Introduction

Energy transfer in the human ear is initiated when sound waves are presented to the ear canal and sound pressure is applied to the tympanic membrane. Energy begins to flow and tympanic membrane vibrates with a characteristic amplitude and phase that depends upon the acoustic admittance of the entire system (Zwislocki, 1963). The transfer of energy from the ear canal to the middle ear and ultimately to the cochlea can be described from measures of sound pressure level (SPL) at the lateral Stoppenbach, 2002).

surface of the tympanic membrane (Wiley and Lucae (1867) was the first to measure tympanic membrane and middle ear characteristics in humans using an instrument called the interference otoscope, by presenting sound into both ears of a participant and listening to the level of sound reflected in each ear canal (cited in Feldmann, 1970). The term impedance was first introduced as an electrical term by Heaviside in 1886 (cited in Heaviside, 1892). Webster extended the principles of electrical impedance laws to the analysis of acoustical system (Webster, 1919). The first acoustic impedance measures made with an actual probe tube inserted into the human ear canal were reported by Troger in 1930 (cited in Metz, 1946). Martin in 1971 reported the first survey of audiologic practices where tympanometry or acoustic immittance measures were not mentioned among the procedures used by practicing audiologists. The recent survey of practicing audiologists reported by Martin et al in 1998 indicated that 96% of the respondents routinely used acoustic immittance measures in their daily practice. Acoustic immittance instruments and procedures are now commonplace in audiology, otolaryngology, pediatric and other diagnostic clinics. The most recent guidelines for screening middle ear function recommended by ASHA (1997) are based on the use of acoustic immittance measures. When it has become such an important tool for our clinical settings, then awareness of the variability in measurements becomes an essentiality for an audiologist. The error most likely to happen is by different placements of probe-tip in the ear canal leading to variability. To verify this hypothesis the present study was conducted.

The hypothesis proposed was based on the principle that accurate determination of the static compliance depends on an accurate estimation of the ear canal volume. If ear canal volumes are overestimated, then the static compliance will be underestimated that could lead to suspicion of middle ear effusion where none exists. Secondly, in the case of flat tympanogram, the estimation of equivalent volume can provide a clue to the cause of the flat tympanogram, whether it is due to artifact, tympanic membrane perforation or patent tympanostomy tube, or middle ear effusion (Fowler CG & Shanks JE, 2002).

II. Material and Methods

The study included twenty adult normal hearing subjects of either sex with age range of 17 to 28 years. Subjects' selection criteria were normal hearing, with no external and middle ear pathology on clinical examination. The impedance audiometry was carried out in sound-treated rooms of Speech and Hearing Unit of ENT OPD, PGIMER, Chandigarh. The equipment

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used for impedance audiometry was SD 30 tympanometer. The 226 Hz probe-tone was used for tympanometric measurements. The pressure in the external auditory canal was varied from +200 to -300 daPa at the rate of 200 daPa per second. The parameters noted were compliance, ear canal volume (or base volume) and middle ear pressure. The acoustic reflex testing was also conducted. The change in compliance denoted the acoustic reflex in the monitored ear with probe tip. The reflexes were elicited and recorded at 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz at 90 dB HL. The change in compliance was also noted from 80 dB HL to 100 dB HL at 1000 Hz. All these parameters were studied in two placement positions of the probe-tip (i) ≤1mm; and (ii) 2mm inside the ear canal to understand the variability.

The data was subjected to appropriate statistical measures. The values of mean for central tendency and standard deviation for variability were computed. Paired t-test was administered for comparison of two positions of probe-tip in the ear canal. The significance tests were two-tailed and conducted at or above the 5% significance level.

The study was conducted in the Speech and Hearing Unit, Department of Otorhinolaryngology, PGIMER, Chandigarh. The investigations were carried out complying the ethics and were approved by the ethical committee of the institute. Each of the subjects was told about the purpose of the investigation and written informed consent was taken from the patients.

III. Observations and Results

A total of twenty subjects with normal hearing and healthy external & middle ear were included in the study. The subjects with age range 17 to 28 years of either sex were assessed for tympanometry and acoustic reflex test in both the ears. Thus total number of ears assessed was forty (40 ears). The table 1 shows that highly significant differences were observed between two positions of the probe-tip for ear canal volume. Differences were also observed for middle ear pressure in two positions but could not reach the statistical significance level. Similar results were seen for compliance differences.

Table 1 : Findings of tympanometry (N= 40)

	1st position (≤1mm)		2nd position (2mm)		't'
Parameters	Mean	SD	Mean	SD	
Compliance(cc.)	0.788	0.427	0.778	0.480	0.639
E C Vol.(cc.)	1.443	0.398	1.147	0.307	**5.989*** **
ME Press. (daPa)	-8.13	20.50	-6.25	16.28	1.292

**p<0.05; **p<0.01; ***p<0.001.*

Table 1 depicts mean, standard deviation (SD) and 't'-value of compliance, ear canal volume (E C Vol.) and middle ear pressure (ME Press.).

Table 2 : Findings of Acoustic Reflex Test (N= 40)

d	1st position (≤1mm)		2nd position (2mm)		t'
Frequency	Mean	SD	Mean	SD	
500 Hz	-6.825	9.142	-6.15	6.100	0.514
1000Hz	-8.975	8.368	-9.400	4.986	0.354
2000Hz	-2.400	3.350	-5.250	9.190	**2.119***
4000Hz	-1.350	2.887	-2.250	4.490	1.630

**p<0.05; **p<0.01; ***p<0.001.*

Table 2 shows mean, standard deviation (SD) and 't'-value of 'change in compliance' during acoustic reflex at 90dB SPL for different frequencies tested.

As seen from the table 2 statistically significant difference was found between two positions of the probe-tip at 2000 Hz. It means that change in compliance during acoustic reflex for 2000 Hz probe-tone was affected by position of the probe-tip in the ear canal. Differences were observed for 4000 Hz probe frequency but could not reach the statistical significance. For 500 Hz and 1000 Hz probe frequencies, the compliance shift with acoustic reflex was again not significantly different in two probe-tip positions.

IV. Discussion

Tympanometry is a measure of the acoustic admittance or compliance in the ear canal as a function of changing ear canal pressure (Wiley TL and Stoppenbach DT, 2002). Tympanometric measures

include contributions offered by the volume of air between the probe-tip and the tympanic membrane as well as the entire middle ear system. The obtained measures will vary with individual ear canal characteristics (e.g. shape and volume) (Wiley TL and Stoppenbach DT, 2002). The probe-tip position thus determines the volume of air in the external ear canal. If the placement of probe-tip is deep in the ear canal the volume would be less and with lateralized placement volume of the ear canal would be greater. Similar findings were found in the present study that when the placement of probe-tip was ≤1mm the ear canal volume was large and with placement position of probe-tip 2mm deep the ear canal volume was smaller. To overcome these differences the compensated measures were recommended by ANSI (ANSI S3.39-1987) that eliminates the contribution of the ear canal to the overall acoustic admittance or compliance (ASHA, 1990). The ear canal volume is not the direct measure but it is the estimate of the admittance or compliance offered by the volume of air between the probe-tip and the tympanic membrane. The measure is based on the principle that under specified conditions, a given volume of air has a specified admittance or compliance (Shanks & Lilly, 1981).

The differences of compliance in two positions were not found statistically significant. The probable reason for this might be the instrument used (Siemens SD 30) where the compliance measured with 226 Hz probe-tone at 200 daPa serves as an approximation of the compensated acoustic admittance of the ear canal.

Similarly there were no significant differences observed for middle ear pressure in two probe-tip positions. Middle ear pressure is an indirect estimate made from the tympanometric peak pressure (TPP) (Wiley TL and Stoppenbach DT 2002), and ANSI defined TPP as the pressure in deca-Pascals at which the peak of the tympanogram occurs (ANSI S3.39-1987). As the subject included in the study were with normal middle ear on examination, thus the middle ear pressure measured should be normal showing non-significant differences in two positions of the probe-tip.

Significant differences were obtained for change of compliance in acoustic reflex testing at 2000 Hz frequency but the differences observed at 4000 Hz could not reach statistical significance. These results can be explained by ear canal resonance principles. The resonant frequency is based on the tube length and is assumed independent of diameter in the normal ear (Goode RL, 2001). The resonant frequency is calculated by the formula F0 = 1/4th wavelength of sound (∂) multiplied with ear canal length. In a normal average ear canal the resonant frequency is about 3500 Hz (= 10cm wavelength) (Goode RL, 2001). In tympanometry the probe-tone used is 226 Hz with longer wavelength hence the F0 would be occurring at ¼th wavelength of sound. Secondly modification in length of ear canal also affects the resonant frequency. As explained by Goode RL et al, 1977 that lengthening the canal lowers the resonant frequency and decreasing the length raises the frequency. In such situation the placement of the probe-tip would either underestimate or overestimate the SPL measured. It would also affect the change in compliance during acoustic reflex.

V. Conclusions

The present study based on data of 40 ears, shows that placement of probe-tip affects the measurements of tympanometry and acoustic reflex testing to some extent. The change of compliance during acoustic reflex is the most affected parameter by shift of probe-tip position during impedance audiometry.

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A Method to Construct an Interim Obturator using Presurgical Tissues for Maxillary Palatal Defect

By Sumeet Sharma, Harvinder Singh, Sarbjeet Singh & Nikhil Dev wazir
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Abstract- The presence of oral cancer necessitates the surgical removal of all or part of the maxilla, leaving the patient with a defect that compromises the integrity and function of the oral cavity. Surgical unit along with the prosthodontic counterpart goes hand in hand for the fulfilment of the post restorative re-establishment of the oral functioning.

The immediate line of treatment includes maxillectomy with the initial insertion of an immediate surgical obturator at the time of surgery followed by the insertion of interim obturator for initial healing which thereafter replaced by definite prosthesis once the tissues are stabilised.

This article will provide a method of fabricating an interim obturator which will be very easy, less time consuming, inexpensive and comfortable for the patient. Material used for this type of obturator is the common self cure acrylic resin duplicating the lost tissues using the preoperative cast.

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I. Introduction

Maxillary cancer surgery often creates a defect that may affect speech, swallowing, mastication, and facial appearance. Prosthetic rehabilitation after total maxillectomy has historically involved the use of maxillary obturator prosthesis[1]. Resection of the hard palate establishes communication between the oral and nasal cavities and often the maxillary sinus[2].

A maxillary obturator prosthesis can re-establish physical separation of the oral cavities[3-4].obturator constructed for maxillectomy patients are grouped according to their stage of use. The surgical obturator is fabricated prior to surgery; the interim obturator prosthesis is constructed removal of the surgical obt - urator and packing, while the definitive obturator prosthesis is provided for the patient 6 to 12 month after surgery[3-4].

Interim obturator prosthesis is normally placed after 7 to 10 days after surgery[5-6]. As healing progresses, interim obturator prosthesis is fabricated and extended further into the defect, with subsequent additions to improve the seal and retention[7]. Artificial replacement of the teeth and palate aids in speech, mastication, esthetics and morale[7-8]. However, the prosthodontist should not rush to provide artificial for the interim obturator prosthesis. The friability of tissue after radiation therapy, if it has been used, usually allows use of only the simplest type of prosthesis[7]. Also posterior teeth should not be added to interim obturator prosthesis since they may impose excessive stress on the wound and delay the healing process[7].

Numerous methods of polymerization and processing are now available and attracted the attention of several investigators[9-10]. Takamata and Setcos[9] reviewed the various modifications of denture base resins and evaluated pourable resin, microwave-polymerized resin, and light activated resins. They found that conventional techniques with heat-activated resins are not only more time consuming, but also may provide reduced accuracy. Takamata et al[11] compared the adaptation of denture base materials processed on a master cast. The greatest discrepancy in adaptation to the master cast occurred with the heat activated resin than self activated resin while the microwave-processed resin provided the best adaptation.

This article describes an easy method to make interim obturator prosthesis more comfortable during the time required for postsurgical healing. The time saved and ease of the procedure, in addition to the use of duplicated artificial teeth, make this technique more economical than the flasking method using heat polymerized acrylic resin and light activated resin. It also provide improved fit and a smoother surface than achieved by other techniques, such as making a matrix with irreversible hydrocolloids and using the pre-existing tissue for duplication before surgery[12].

II. Technique

1. A presurgical cast is made using a type II dental plaster with the water: powder ratio of 0.50 as recommended and a final cast is made after maxillectomy using dental stone IV(Denflo extra hard die stone;prevest DenPro®,India) with water: powder ratio of 0.24 as recommended.(Fig.1 and Fig. 2)

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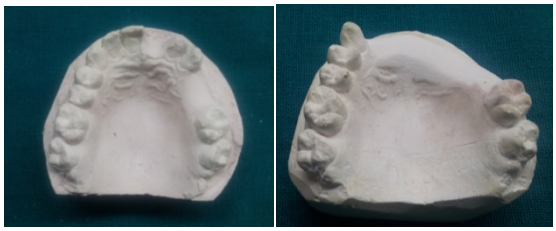

Figure 1 : Presurgical maxillary cast

Figure 2 : Postsurgical Maxillary cast

2. On the presurgical cast, prefabricated acrylic teeth (Rolex cross linked acrylic teeth, India) are arranged if there is any edentulous area exists.(Fig.3).

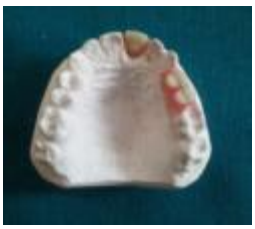

Figure 3 : Arranged acrylic teeth in edentulous areas

3. On the presurgical cast, remove one by one the teeth which in later stage is planned to be extracted during the maxillectomy procedure.
4. Arrange prefabricated acrylic teeth on the edentulous area duly created by removal of anterior teeth.(Fig.4)

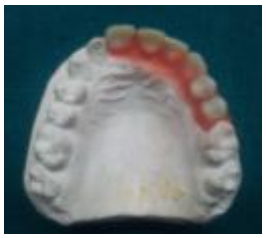

Figure 4 : Acrylic teeth placed following removal of natural teeth

5. Adapt a shellac base plate wax (Hiflex shellac base plate; Prevest DenPro®,India) with the margin extended till the interproximal gingival level of remaining natural teeth and flushing with the lingual cervical margins of the acrylic teeth. (Fig. 5)

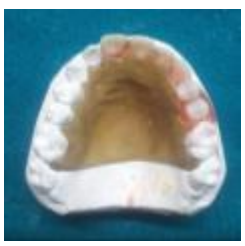

Figure 5 : Shellac base plate adapted

6. Seal the shellac base plate wax using sticky wax to prevent any dislodgement during impression making.
7. Once the teeth and the shellac base plate wax is secured on the presurgical cast, a putty impression material (Affinis; Coltene Whaledent, Cayahoga, Ohio) is then adapted over the cast which includes all the arranged acrylic teeth, the palatal area, remaining natural teeth and the anterior sulcus.
8. A stainless steel hollow cylindrical mould with the dimension of 25mm length and 10mm width is then inserted on the palatal portion on the adapted putty impression material which will facilitate the ingress of the acrylic resin. (Fig.6)

Figure 6 : Stainless steel mould inserted in palatal portion

9. Remove the putty matrix from the presurgical cast. Arrange the acrylic resin teeth in the respective indentation made in the putty matrix.
10. Secure the acrylic teeth with the matrix using a cyanoacrylate. (Fig.7)

Figure 7 : Acrylic teeth secured in place

11. Adapt Co-Cr wire (Sun- Cobalt Clasp wire; Dentsply, Tochigi, Japan) clasps to the teeth on the postsurgical cast to retain and stabilize the prosthesis and secure the tags of the clasp using cyanoacrylate and coat the cast by painting separating medium with a brush.(Fig. 8)

Figure 8 : Clasps adapted

12. Adapt the putty matrix on the postsurgical cast using the reference of natural teeth and the buccal tissues.
13. Secure the putty matrix periphery with the tissues using a sticky wax.(Fig. 9).

Figure 9 : Periphery secured and blocked with sticky wax

14. Pour a liquid mix of clear autopolymerizing resin (Palapress vario; Heraeus Kulzer Co) at a powder ; liquid ratio of 10g to 7 ml through the stainless steel mould attached to the matrix (Fig.10)

Figure 10 : Clear autopolymerizing resin is poured through mould

15. Place the cast with the resin during dough stage in a pressure pot with water. Heat the water gradually from room temperature to 45 degrees celcius, at 2-bar pressure for 30 mins12, to harden and reduce porosity of acrylic resin.
16. Once the resin is set, remove the putty matrix from the cast and evaluate the teeth and palatal portion duplicated in acrylic resin.
17. Remove the prosthesis from the cast. Trim the excess acrylic resin with carbide bur (Laboratory Cardibe bur; Mani) and polish the prosthesis with finishing bur and waterproof abrasive paper conventionally.12 (Fig.11)

Figure 11 : Finished Obturator

III. Summary

Effective obturation of the unilateral or bilateral maxillectomy defect is a difficult task for the maxillofacial prosthodontist. Multidisciplinary appr-oach to the treatment is essential to achieve adequate retention and function for the surgical obturator prosthesis. Duplication of the presurgical contours of the teeth and palatal tissue in interim obturator prosthesis may facilitate speech and deglutition and also improve estetics of the patient. This technique of making obturator prosthesis permits the immediate replacement of postoperative anterior teeth and maxillary palatal form. This method of fabrication not only reduces the time consumed during fabrication also helps in rehabilitation of patient undergoing maxillectomy in an expedious and non-traumatic manner. This kind of method is limited to a lesser extent of the tissue loss whereas; when the extent of the maxillectomy is deeper a hollow bulb type of obturator is more preferable.

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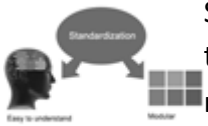

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2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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