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Immediate implant placement into dentoalveolar sockets in conjunction with plasma rich in growth factor.

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INDEX

1. INTRODUCTION

Tooth loss and edentulism were common among older people just a few decades ago, meanwhile nowadays this tendency is changing and recent studies have yielded a significant decline in edentulism and tooth loss in adult and older populations as well as among subcategories of elderly persons such as the oldest old (Petersen, 2005; Vilstrup 2007; Muller 2007).

The decision to extract teeth is not only influenced by the extent of caries and its sequelae and/ or periodontal disease, but is also based on the value placed on tooth retention by dentists and patients and the patients' ability to pay for dental treatments (Holm-Pedersen 2007). This suggests that tooth loss may also be related to complex behavioural and socio-economic factors (Joshipura 2005).

From a pathologic point of view tooth loss could occur due to the sequent reasons: periodontal disease, caries, alveolar trauma and fracture, vertical root fracture, cracks and endodontic lesion.

Periodontal disease

Periodontal disease are considered one of the main causes of tooth loss.

According to the Seventh European Workshop on Periodontology "Periodontal diseases are the pathological manifestation of the host response against the bacterial challenge from the dental biofilm at the tooth/gingival interface". Periodontal diseases are divided in two main categories:

1. Plaque-induced gingivitis is a chronic inflammatory response to the accumulation of supragingival biofilm.
2. Periodontitis is a chronic inflammatory disease that results from a complex polymicrobial infection, leading to tissue destruction as a consequence of the perturbation of the homeostasis between the subgingival microbiota and the host defences in susceptible individuals. This bacterially driven disease may be considered to differ from an accepted definition of infection (i.e. invasion by and multiplication of pathogenic microorganisms in a body part or tissue which may produce subsequent tissue injury and progress overt disease through a variety of cellular or toxic mechanisms). (Sanz 2011)

The prevalence of periodontitis is very difficult to estimate due to the wide range of studies published. There is a conspicuous lack of uniformity in the definition of periodontitis used in epidemiological studies, and findings from different research groups are not readily interpretable (Borrell 2005).

By definition periodontitis when not treated leads to tooth loss. Again, it is difficult to estimate the survival rate of the periodontal compromised dentition. It's obviously unethical observing the progression of an untreated disease.

Otherwise it's possible to evaluate the longevity of the periodontally compromised tooth on the basis of cohort studies performed to assess the efficacy of periodontal therapy (Holm-Pedersen 2007).

Tonetti et al evaluated during the course of up to 22 years of treated and well-maintained periodontal patients, 0.23 compromised teeth were lost per patient per year, i.e. one tooth was lost every 5 years in

this population treated for advanced periodontitis. The teeth lost were predominantly molar teeth (Tonetti, 2000).

Other studies have investigated the survival rate of periodontally compromised teeth with furcation involvement. The overall survival rate was > 90% after 10 years of follow up. The survival rate decreased when the periodontally compromised teeth were also endodontically treated (Holm-Pedersen 2007).

Caries

The caries process is initiated in the biofilm or dental plaque (Fejerskov 1990; Manji 1991; Fejerskov 1994). Biofilms form on any solid surface exposed to appropriate amounts of water and nutrient (Wimpenny 1994). The dental tissues, enamel, dentin, and cementum, are the relevant oral solid surfaces, and these surfaces are coated by a pellicle to which the microbial cells attach. The primary colonizers and secondary organisms generate a matrix of exopolymer within which cells grow. A community of organisms is formed rather than a haphazard collection of bacteria. The community has a collective physiology which can solve the specific physicochemical problems posed by the environment at the site.

The bacteria in the biofilm are always metabolically active, causing fluctuations in pH. These fluctuations may cause a loss of mineral from the tooth when the pH is dropping or a gain of mineral when the pH is increasing (Manji F, 1991). The cumulative result of these de- and re-mineralization processes may be a net loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion.

The biofilm tends to form and mature in certain locations on the tooth, notably the occlusal surface, especially during eruption, the approximal surface cervical to the contact point, and along the gingival margin. These areas are relatively protected from mechanical wear by tongue, cheeks, abrasive food, and toothbrushing. Thus, these are the sites where caries lesions may become visible. It should be noted, however, that there is nothing special chemically about these particular areas of the tooth surface—they are susceptible to lesion development only because the biofilm tends to stagnate there and remain "undisturbed" for prolonged periods of time.

Root caries is similar to enamel caries in being a subsurface demineralization, but, unlike enamel caries, the surface may appear softened at an early stage of lesion development. Bacteria penetrate at an earlier stage than in coronal caries. Even when microcavities are observed, the demineralization is deep to a relatively well-mineralized surface layer. Interestingly, although these lesions may appear rather extensive, they are seldom more than 0.5 to 1 mm deep. This slow rate of bacterial invasion and tissue degradation gives the opportunity for these lesions to be arrested by plaque control with fluoride toothpaste (Nyvad 1986; Nyvad 1997). Once again, it is the biofilm at the surface of the lesion that is driving the process.

Secondary or recurrent caries is primary caries at the margin of a restoration (Mjör and Toffenetti 2000). The histological picture will show primary caries next to the restoration margin, and there may be lines of demineralization, called wall lesions, running along the cavity wall. These are a consequence of microleakage, but clinical and microbiological studies appear to indicate that this leakage does not lead to active demineralization beneath the restoration. (Kidd & Fejerskov 2004).

Caries can lead to tooth extraction as we can see from systematic review from Sailer et al (Sailer 2007) which analysed the survival rate of fixed dental prostheses (both metal-ceramic and full-ceramic restorations).

The incidence of caries at abutments was 4.8% for the metal ceramic and 1.8% for full ceramic and 1,6% and 1,7 respectively of the abutments were lost due to caries.

An extensive carious lesion that extends beyond or to the level of the alveolar bone usually represents a challenge for the clinician in restorative terms and a substantial increase in treatment costs for the patient. If a tooth is restorable, orthodontic extrusion, crown lengthening, or muco-gingival surgical procedures are usually necessary to respect the biologic width (Tseng 1997).

Endodontic lesion

The periradicular infection are induced by microbial agents which are essential to the progression and perpetuation of this inflammatory diseases (Kakehashi 1965 - Moller 1966 - Sundqvist 1976 – Moller 1981).

Infection occurs after the necrosis of the pulp and a restricted set of microbial species is more prevalent in different forms of periradicular diseases.

There is a lot of evidence regarding microrgansim which play a primary role in the aetiology of periradicular disease.

Evidence suggests that a consortium, not a particular species, possesses the physiologic requirements necessary to cause damage to the periradicular tissues. In addition, it is becoming apparent that different compositions of the root canal microbiota can possess equal ability to elicit tissue injury. Specific mixtures of species that are implicated in the pathogenesis of periradicular diseases are still unknown, but it is conceivable that the most frequently isolated species may make a major contribution to the ecology of the community colonizing the root canal system and consequently to the degree of pathogenicity of the consortium. Some of these most prevalent species, not all, exert an important role in pathogenicity and thereby act as key pathogens. (Siqueira, 2002).

The persistent presence of microorganisms (after primary endodontic treatment) which form a complex aggregate within the root canals is the cause of secondary periapical lesions. Apically secondary lesion is defined as "the presence of a periradicular inflammatory lesion (apical or lateral) in an endodontically treated tooth when the lesion is no longer can be assumed to be undergoing healing after the root canal treatment" (Haapasalo 2011).

There are difference in the composition of the microbiota related to the type of infection, if it is primary infection with the necrosis of the pulp or is a secondary apical periodontitis. Gomes et al (2004) investigated this issue taking samples from 60 root canals, 41 with necrotic pulp tissues (primary infection) and 19 with failed endodontic treatment (secondary infection). The root canal microflora of untreated teeth with apical periodontitis was found to be mixed, comprising gram-negative and gram-positive and mostly anaerobic microorganisms and usually containing more than 3 species per canal. On the other hand, facultative anaerobic and gram-positive bacteria predominated in canals with failed endodontic treatment, which harbored 1±2 species per canal.

A recent longitudinal study in the United States reports a survival rate of 94% of the endodontically treated teeth (Salehrabi 2004.)

A systematic review by Del Fabbro et al (2008) compared surgical versus non surgical retreatment for periradicular lesions. They analysed three randomised controlled trial and found non significant difference between these two techniques. he choice between a surgical and a non-surgical procedure should rely upon factors other that the mere treatment outcome: these factors should include patient's initial clinical situation, patient's preference, operator's experience and skill, com- plication risk, technical feasibility, and overall cost.

Despite to this high survival rate it's interesting to focus on the causes of the failure or in other words why some endodontically treated teeth can not be retreated (orthograde re-treatment or surgical treatment) and need to be extracted.

Fuss et al (Fuss, Z, 1999) found that the reasons were related to the quality of the crown restoration (43.5%), the endodontic treatment (40.2%), a vertical root fracture (10.9%), and periodontal diseases (5.5%). Vire (1991) noted in a study of 116 endodontically treated teeth that in 59% of the cases, prosthodontics reasons motivated the extraction, followed by periodontal disease (32%) and endodontic treatment failure (9%). In another study, Zadik et al (2008) found the following reasons: nonrestorable caries 61.4%, endodontic treatment failure 12.1%, vertical root fracture 8.8%, iatrogenic perforations and stripping 8.8%, periodontal diseases 4.6%, cusp fractures 2.4%, orthodontic factors 1.3%, prosthetic factors 0.2%, and trauma 0.5%. (Toure 2011).

Fracture and cracks

Dental trauma are a relatively common event, especially in male under 19 (Glendor 2008).

Dental trauma could result in tooth fracture, which can be horizontal or longitudinal.

Complicated horizontal tooth fracture involve the enamel, the dentin and the pulp chamber (Andreasen 2007, Cohen 2008, Flores 2007). the incidence of this fracture is between 5 and 8% of every traumatic dento-alveolar lesion.

If the tooth maintains his vitality, the first treatment choice consists in the direct covering of the pulp with Mineral Trioxide Aggregate (MTA). The success rate of this treatment ranges between 63 to 88% (Taschieri 2011).

The fracture that occur primarily in vertical plane are divided in five categories (Rivera 2009): craze lines, fractured cusp, cracked tooth, split tooth and vertical root fracture.

The incidence of these kind of fracture is difficult to estimate due to an of then complicated and unclearly diagnosis. Surely the incidence is increased over recent years because patients are aging, with a decrease in tooth extraction. The internal strength of the teeth is compromised from complex restorative and endodontic procedure which remove dentin (Kishen 2006).

The *craze lines* are common in adults; hey extend over marginal ridges, buccal and lingual surfaces in posterior teeth, and as long vertical defects in anteriors. Only the enamel is involved and is a natural occurrence (Rivera 2009).

The *fractured cusp* is a complete or incomplete fracture initiated from the crown of the tooth and extending subgingivally, usually directed both mesio-distally and facio-lingually; the fracture usually involves at least two aspects of the cusp by crossing the marginal ridge and also extending down a facial or lingual groove. The fracture will extend to the cervical third of the crown or root. Fractured cusps are relatively easy to diagnose and treat and usually have a good prognosis (Rivera 2009).

An unsupported cusp, due to a large restoration or an extensive carie, has the highest risk to fracture itself.

Cracked tooth is an incomplete fracture initiated from the crown and extending subgingivally, usually directed mesio-distally (Hiatt 1973, Cameron 1964, Cameron 1976). The fracture may extend through either or both of the marginal ridges and through the proximal surfaces. The fracture is located in the crown portion of the tooth only, or may extend from the crown to the proximal root. Otherwise to fracture cusp, this kind of fracture is centred more occlusally and more apically, which makes worse its prognosis.

Split tooth is a complete fracture initiated from the crown and extending subgingivally, usually directed mesio-distally through both of the marginal ridges and through the proximal surfaces. The fracture is located coronally and extends from the crown to the proximal root. It is an evolution of a cracket tooth: tooth segments are now entirely separated. These fracture usually include the pulp. This kind of fracture is more common in root canal-treated teeth (Rivera 2009).

Vertical root fracture (VRF) is defined as a complete or incomplete fracture initiated from the root at any level, usually directed facio-lingually (Rivera 2009).

VRF usually originates from the apical end of the root and propagate coronally or can originate from the cervical portion of the root with extension in an apical direction *(*American Association of Endodontists, 1997, Rundquist 2006*)*

In a horizontal aspect, VRF expands laterally from the root canal wall to the root surface where it may result in an incomplete fracture involving only one side of the root

A complete fracture expands in opposite directions of the root canal and involves two root surface aspects (Walton 1984).

The VRF also involve the surrounding tissue in its development. Due to the communication to the gingival sulcus an inflammatory process begins around the fracture and involves periodontal tissue (Walton 1984) and leading to periodontal ligament breakdown, alveolar bone loss, and granulation tissue formation (Bergenholtz G, 2003).

It results in a bone dehiscence as described by Lustig in the buccal plate in 90% of the examined cases (Lustig 2000).

From a clinical point of view the diagnosis is not easy.

The patient can show a teeth with a sinus tract (if two sinus tract is a pathognomonic sign), a periodontal abscess and a deep osseous defect.

A deep osseous defect is quite typical sign because its development. Initially is narrow and difficult to probe, just in face where the fracture line is. At a later stage when the defect has extended laterally, is easier to probe. To differentiate this sign from periodontal probing, it has to be noted that fracture involve only isolated areas. It is typical to probe physiologically (1, 2 mm) for example in mesial, distal and lingual side, while buccally, in a restricted area, there is a pocket of 10 mm.

Clinical signs and symptoms as well as radiographic presentations are often similar to those associated with non-healing root canal treatments and with certain manifestations of periodontal disease.

Therefore it is difficult to estimate the prevalence of VRF. Reports from case series (Meister 1980) and follow-ups of patients treated with prosthetic reconstructions (Torbjorner 1995 - Bergman 1989), and retrospective radiological studies (Morfis 1990) suggest a prevalence of 2% and 5% (Tamse, 2006)

Alveolar trauma

Alveolar trauma could be associated with or without dental trauma. Dental trauma derived from alveolar trauma are: coronal fracture, root coronal fracture, luxation, completely extraction of the tooth (Flores 2007).

The last two categories are the most represented, especially regarding trauma in the anterior maxillary bone.

Alveolar trauma could be complete (with entirely detachment of bone chip) or incomplete (when the bone is totally retained).

Bone chips could affect and lacerate soft tissue in the traumatised area, and the occlusion coul be changed by the migration of the teeth.

The treatment includes the positioning and fixing of the bone fragment dislocated. Teeth should be splinted. In every case, radiographic and clinical controls should last almost five years.

Luxation

Following the classification of the International Association of Dental Traumatology (DiAngelis 2012), there are five types of luxations injuries. *Concussion* is when the tooth is tender to touch or tapping; it

has not been displaced and does not have increased mobility. Sensibility tests are likely to give positive results.

Subluxation when The tooth is tender to touch or tapping and has increased mobility; it has not been displaced. Bleeding from gingival crevice may be noted and sensibility testing may be negative initially indicating transient pulpal damage. It is important to monitor pulpal response until a definitive pulpal diagnosis can be made. *Extrusive luxation* is when the tooth appears elongated and is excessive mobile; sensibility tests will likely give negative results.

Lateral luxation is when the tooth is displaced, usually in a palatal/lingual or labial direction; It is immobile and percussion usually gives a high, metallic (ankylotic) sound. Fracture of the alveolar process is present and sensibility tests are negative.

Intrusive luxation is when the tooth is displaced axially into the alveolar bone. It is immobile, and percussion may give a high, metallic (ankylotic) sound. Sensibility tests are negative.

Luxations are between 30 and 44% of dento-alveolar trauma (Cohen 2008).

Avulsion of permanent tooth

When there is avulsion due to an injury, permanent tooth is out of his socket in its entirely. All the support system is broken and the prognosis is very complicated because the possibility of biological complication

Avulsions of permanent teeth have an incidence between 1 to 16%, especially in young people (8 and 12 years old). In these early phase, the tooth is not totally formed(Andreasen 2007, McIntyre 2009).

Replace a tooth

After tooth extraction patient can choose different treatment options to replace missing tooth or teeth.

With the new possibilities in terms of materials and the introduction of implantology as daily practice, a fixed solution to substitute the missing tooth (or teeth) can be offered.

Dental implants are a consolidated treatment for replacing missing teeth, allowing restoration of chewing function, speech, and aesthetics. Implants are inserted into the jawbones to support a dental prosthesis and are retained because of the intimacy of bone growth on to their surface. Such direct structural and functional connection between living bone and implant surface, termed osseointegration, has surely been one of the most significant scientific breakthroughs in dentistry over the past 30 years.

Pjeturrson and Lang (2008) propose a prosthetic treatment planning on the basis of scientific evidence.

For a *single tooth gaps in the anterior maxilla* an implant supported single crown if the adjacent teeth are intacts is the most conservative and most biological treatment option. This option has the lowest failure rate too.

When the *single tooth gap is in the mandible* this option is still the better one also because the preparation of mandibular incisors presents a serious risk for the pulp vitality.

In the posterior region (both maxilla and mandible) the implant supported single crown is the first choice choice and represents the most tissue-preserving treatment. The conventional FDP should only be chosen if the adjacent teeth require reconstructions.

If multiple adjacent teeth are lost in the anterior maxilla or mandible, two non adjacent implants could be the better solution. However the clinician has to evaluate the volume of soft tissues and bone loss which occurred. The alternative is fixed dental prostheses , which have a slightly higher survival rate when compared with implant supported restoration at 10-years follow up (Pjetursson 2004.) (Pjetursson 2007)

In the posterior region when multiple missing teeth have to be replaced the implant supported fixed prostheses is the first choice due to the short extension of the reconstruction itself.

This solution permits to reduce the biomechanical risks of a long span conventional FDP, although technical complication are more frequent (Pjetursson 2007).

The implant supported restoration is preferred again in case of molar extraction when compared to a tooth-supported cantilever Fixed Dental Prosheses, especially if non vital endodontically treated terminal-abutments are involved, due to increasingly risk of fracture (Nyman 1979) (Hammerle 2000).

2. Aim

The purpose of this thesis is to investigate the perspective to rehabilitate an infected socket healing with a post-estractive implant and the adjunct of Plasma Rich in Growth Factors.

The natural healing process after tooth extraction normally manages residual infection, but as an infection increases inflammatory activity, infection may result in increased bone resorption and a higher risk of implant stability loss and failure. When a tooth is lost due to endodontic or periodontal infection the residual socket is contaminated by bacteria. A granulation tissue is present as a reactive tissue to protect the bone from direct bacterial aggression. (Villa & Rangert 2007)

Traditionally, before placing dental implants, the compromised teeth are removed and the extraction sockets left to heal for several months to 1 year. This conservative approach however let the bone resorption to advance. Following the correct clinical indications, early placement of the implants into the extraction sockets might avoid this undesirable resorption (Werbitt 1992 - Schropp 2003).

Also, the treatment time is reduced and the patients undergoes to only one surgical procedure.

The immediate post-extraction implant placement allow the clinicians to preserve aesthetics, to reduce the total treatment time, to maintain the socket wall, to reduce the operative time (Lazzara RJ 1993)

Otherwise placement implant in an infected post-extractive socket is still a controversial challenge, and there are few studies treating this topic. (Del Fabbro 2009).

In contrast to this procedure there is the residual infection of the socket which could contaminates the implant surface. Nevertheless in orthopaedic surgery vertebral osteomyelitis are successfully treated with meticulous bone debridement and antibiotic therapy combined with titanium mesh cages that provide immediate support and stability for the weakened vertebrae (Hee HT 2003).

This findings can be applied in dentistry with implants into fresh extraction sites with chronic periapical pathology.

Autologous platelet concentrate are widely used in oral surgery to enhance the healing and post-operative progress. A recent literature review has stated that favourable effects on hard and soft tissue healing and postoperative discomfort reduction were often reported in post-extraction socket healing (Del Fabbro 2011).

Plasma Rich in Growth Factors (PRGF) is characterized by a more sustained release of growth factors as calcium chloride instead of thrombin is used (Tsay 2005), a moderated platelet concentration which has been related with optimal biological benefit (Weibrich, 2004) and the formation of a three-dimensional fibrin scaffold which maintains the regenerative space and serves as matrix for progenitor cells. Leukocyte content has been eliminated from PRGF with the aim of avoiding the pro-inflammatory effects of the proteases and acid hydrolases contained in white blood cells (Schnabel 2007).

Bibliography

- Andreasen et al Textbook and color atas of traumatic injuries to the teeth, 4th edition Blackwell Munksgaard, Oxford 2007
- Barzilay I. Immediate implants: Their current status. Int J Prosthodont. 1993 Mar-Apr;6(2): 169-75.
- Bergenholtz G, Hasselgren G. Endodontics and periodontics. In: Lindhe J., ed. Clinical Periodontology and Implant Dentistry, 4th edn. Blackwell Munksgaard, Copenhagen: 2003: 318–351
- Bergman B, Lundquist P, Sjogren U, Sundquist G. Restorative and endodontic results after treatment with cast posts and cores. J Prosthet Dent. 1989 Jan;61(1):10-5.
- Borrell LN, Papapanou PN. Analytical epidemiology of periodontitis. J Clin Periodontol. 2005;32 Suppl 6:132-58.
- Cameron CE. Cracked-tooth syndrome.J Am Dent Assoc. 1964 Mar;68:405-11.
- Cameron CE. The cracked tooth syndrome: additional findings. J Am Dent Assoc. 1976 Nov; 93(5):971-5.
- Cohen S., Burns RC.: Pathways of the pulp, 8th edition ed Mosby, St Lois 2008
- Del Fabbro M, Taschieri S, Testori T, Weinstein R. Surgical versus non-surgical endodontic re-treatment for periradicular lesions, The Cochrane Library 2008, Issue 4
- Del Fabbro, M., Boggian, C., Taschieri, S Immediate Implant Placement Into Fresh Extraction Sites With Chronic Periapical Pathologic Features Combined With Plasma Rich in Growth Factors: Preliminary Results of Single-Cohort Study. J Oral Maxillofac Surg. 2009 Nov;67(11): 2476-84.
- Del Fabbro, M, Bortolin, M, Taschieri, S Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. Int J Oral Maxillofac Surg. 2011 Sep;40(9): 891-900.
- Diangelis AJ, Andreasen JO, Ebeleseder KA, Kenny DJ, Trope M, Sigurdsson A, Andersson L, Bourguignon C, Flores MT, Hicks ML, Lenzi AR, Malmgren B, Moule AJ, Pohl Y, Tsukiboshi M. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxations of permanent teeth. Dent Traumatol. 2012 Feb;28(1):2-12
- Endodontics: Colleagues for excellence. Cracking the Cracked Tooth Code. American Association of Endodontists, 1997:Chicago
- Fejerskov O, Manji F (1990). Risk assessment in dental caries. In: Risk assessment in dentistry. Bader JD, editor. Chapel Hill: University of North Carolina Dental Ecology, pp. 215-217
- Fejerskov O, Thylstrup A (1994). Different concepts of dental caries and their implications. In: Textbook of clinical cariology. 2nd ed. Thylstrup A, Fejerskov O, editors. Copenhagen: Munksgaard, pp. 259- 283

- Flores MT, Andersson L, Andreasen JO, Bakland LK, Malmgren B, Barnett F, Bourguignon C, DiAngelis A, Hicks L, Sigurdsson A, Trope M, Tsukiboshi M, von Arx T; International Association of Dental Traumatology. Guidelines for the management of traumatic dental injuries. I. Fractures and luxations of permanent teeth. Dent Traumatol. 2007 Apr;23(2):66-71.
- Fuss Z, Lustig J, Tamse A. Prevalence of vertical root fractures in extracted endodontically treated teeth. Int Endod J. 1999 Aug;32(4):283-6.
- Glendor U. Epidemiology of traumatic dental injuries--a 12 year review of the literature. Dent Traumatol. 2008 Dec;24(6):603-11.
- Gomes BP, Pinheiro ET, Gadê-Neto CR, Sousa EL, Ferraz CC, Zaia AA, Teixeira FB, Souza-Filho FJ Microbiological examination of infected dental root canals. Oral Microbiol Immunol. 2004 Apr;19(2):71-6.
- Haapasalo M, Ya Shen, Ricucci D Reasons for persistent and emerging post-treatment endodontic disease. Endodontic Topics. 2011 18, 31–50
- Hammerle CH, Ungerer MC, Fantoni,PC, Bragger U, Burgin W, Lang NP. Long-term analysis of biologic and technical aspects of fixed partial dentures with cantilevers.Int J Prosthodont. 2000 Sep-Oct;13(5):409-15.
- Hee HT, Majd ME, Holt RT, Pienkowski D Better treatment of vertebral osteomyelitis using posterior stabilization and titanium mesh cages. J Spinal Disord Tech. 2002 Apr;15(2):149-56;
- Hiatt WH. Incomplete crown-root fracture in pulpal– periodontal disease. J Periodontol. 1973 Jun;44(6):369-79.
- Holm-Pedersen P, Lang NP, Muller F. What are the longevities of teeth and oral implants? Clin Oral Implants Res. 2007 Jun;18 Suppl 3:15-9.
- Joshipura, KJ, Ritchie, C. Commentary to paper: Can the relationship between tooth loss and chronic disease be explained by socio-economic status? Eur J Epidemiol. 2005;20(3):203-4.
- Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965 Sep; 20:340-9.
- Kidd, E. A. M., Fejerskov, O. What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms. J Dent Res. 2004;83 Spec No C:C35-8.
- Kishen A. Mechanisms and risk factors for fracture predilection in endodontically treated teeth. Endod Topics 2006: 13: 57–83..
- Lazzara RJ. Immediate implant placement into extraction sites: Surgical and restorative advantages. Int J Periodontics Restorative Dent. 1989;9(5):332-43.
- Liljenqvist U, Lerner T, Bullmann V, Hackenberg L, Halm H, Winkelmann W. Titanium cages in the surgical treatment of severe vertebral osteomyelitis. Eur Spine J. 2003 Dec;12(6):606-12.

- Lustig JP, Tamse A, Fuss Z. Pattern of bone resorption in vertically fractured endodontically treated teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000 Aug;90(2):224-7.
- Manji F, Fejerskov O, Nagelkerke NJD, Baelum V. A random effects model for some epidemiological features of dental caries. Community Dent Oral Epidemiol. 1991 Dec;19(6): 324-8.
- McIntyre JD, Lee JY, Trope M, Vann WF Jr. Permanent tooth replantation following avulsion: using a decision tree to achieve the best outcome. Pediatr Dent. 2009 Mar-Apr;31(2):137-44.
- Meister F, Lommel TJ, Gerstein H. Diagnosis and possible causes of vertical root fractures. Oral Surg Oral Med Oral Pathol. 1980 Mar;49(3):243-53.
- Mjör IA, Toffenetti F. Secondary caries: a literature review with case reports. Quintessence Int. 2000 Mar;31(3):165-79.
- Möller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981 Dec;89(6): 475-84.
- Mo ller AJR. Microbial examination of root canals and periapical tissues of human teeth. Odontologisk Tidskrift 1966; 74(suppl)
- Morfis AS. Vertical root fractures. Oral Surg Oral Med Oral Pathol. 1990 May;69(5):631-5.
- Muller F, Naharro, M, Carlsson, G.E. What are the prevalence and incidence of tooth loss in the adult and elderly population in Europe? Clin Oral Implants Res. 2007 Jun;18 Suppl 3:2-14.
- Nyman S, Lindhe J. A longitudinal study of combined periodontal and prosthetic treatment of patients with advanced periodontal disease. J Periodontol. 1979 Apr;50(4):163-9.
- Nyvad B, Fejerskov O. Active root surface caries converted into inactive caries as a response to oral hygiene. Scand J Dent Res. 1986 Jun;94(3):281-4.
- Nyvad B, ten Cate JM, Fejerskov O. Arrest of root surface caries in situ. J Dent Res. 1997 Dec; 76(12):1845-53.
- Petersen, PE, Yamamoto, T. Improving the oral health of older people: the approach of the WHO Global Oral Health Programme.Community Dent Oral Epidemiol. 2005 Apr;33(2):81-92
- Pjetursson BE, Bragger U, Lang NP, Zwahlen M. Comparison of survival and complication rates of tooth supported fixed partial dentures and implant supported fixed partial dentures and single crowns. Clin Oral Implants Res. 2007;18(Suppl. 3):97–113.
- Pjetursson BE, Bragger U, Lang NP, Zwahlen M. Comparison of survival and complication rates of tooth-supported fixed dental prostheses (FDPs) and implant-supported FDPs and single crowns (SCs). Clin Oral Implants Res. 2007 Jun;18 Suppl 3:97-113.
- Pjetursson BE, Tan K, Lang NP, Bragger U, Egger M, Zwahlen M. A systematic review of the survival and complication rates of fixed partial dentures (FDPs) after an observation period of at least 5 years – I. Implant supported FDPs. Clin Oral Implants Res. 2004 Dec;15(6):625-42.

- Rivera, Walton Longitudinal tooth fractures: findings that contribute to complex endodontic diagnoses Endodontic Topics 2009, 16, 82–111
- Rundquist BD, Versluis A. How does canal taper affect root stresses? Int Endod J. 2006 Mar;39 (3):226-37.
- Sailer I, Pjetursson BE, Zwahlen M, Hämmerle CHF. A systematic review of the survival and complication rates of all-ceramic and metal-ceramic reconstructions after an observation period of at least 3 years. Part II: fixed dental prostheses. Clin Oral Implants Res. 2007 Jun;18 Suppl 3:86-96.
- Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. J Endod. 2004 Dec;30(12):846-50.
- Sanz M, van Winkelhoff AJ; Working Group 1 of Seventh European Workshop on Periodontology. Working Group 1 of Seventh European Workshop on Periodontology. Periodontal infections: understanding the complexity--consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011 Mar;38 Suppl 11:3-6.
- Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, Fortier LA.. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res. 2007 Feb;25(2):230-40.
- Schropp L, Kostopoulos L, Wenzel A. Bone healing following immediate versus delayed placement of titanium implants into extraction sockets: A prospective clinical study. Int J Oral Maxillofac Implants. 2003 Mar-Apr;18(2):189-99.
- Siqueira JF Jr. Endodontic infections: Concepts, paradigms, and perspectives, Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Sep;94(3):281-93.
- Sundqvist G. Bacteriological studies of necrotic dental pulps [dissertation]. Umea (Sweden): University of Umea; 1976
- Tamse, A. Vertical root fractures in endodontically treated teeth: diagnostic signs and clinical management. Endodontic Topics. 2006 13(1), 84–94. Wiley Online Library.
- Taschieri T, Orlando B, Derchi G, Corbella S. Traumi e Fratture Dento Alveolari, cap 19 pp835-68 in Manuale di Chirurgia Orale, Società di Chirurgia Orale, 2011, Elsevier, Milano.
- Tonetti MS, Steffen P, Muller-Campanile V, Suvan J, Lang NP. Initial extractions and tooth loss during supportive care in a periodontal population seeking comprehensive care. J Clin Periodontol. 2000 Nov;27(11):824-31.
- Torbjörner A, Karlsson S, Odman PA. Survival rate and failure characteristics for two post designs. J Prosthet Dent. 1995 May;73(5):439-44.
- Touré B, Faye B, Kane AW, Lo CM, Niang B, Boucher Y. Analysis of Reasons for Extraction of Endodontically Treated Teeth: A Prospective Study. J Endod. 2011 Nov;37(11):1512-5.
- Tsay RC, Vo J, Burke A, Eisig SB, Lu HH, Landesberg R. Differential growth factor retention by platelet rich plasma compo- sites. J Oral Maxillofac Surg. 2005 Apr;63(4):521-8.

- Tseng CC, Yuan K, Wang WL, Chen YH, Huang CC, Wolff LF. Simultaneous team approach of a crown- lengthening procedure and an operative restoration: Technique and long-term effect. Quintessence Int. 1997 Apr;28(4):249-54.
- Villa R, Rangert B. Immediate and early function of implants placed in extraction sockets of maxillary infected teeth: A pilot study. J Prosthet Dent. 2007 Jun;97(6 Suppl):S96-S108.
- Vilstrup L., Holm-Pedersen P, Mortensen EL, Avlund K. Dental status and dental caries in 85-year-old Danes. Gerodontology. 2007 Mar;24(1):3-13.
- Vire DE. Failure of endodontically treated teeth: classification and evaluation. J Endod. 1991 Jul;17(7):338-42.
- Walton RE, Michelich RJ, Smith GN. The histopathogenesis of vertical root fractures. J Endod. 1984 Feb;10(2):48-56.
- Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 2004 Apr;34(4):665-71.
- Werbitt MJ, Goldberg PV. The immediate implant: Bone preservation and bone regeneration. Int J Periodontics Restorative Dent. 1992;12(3):206-17.
- Wimpenny JWT (1994). The spatial organisation of biofilm. In: Bacterial biofilms and their control in medicine and industry. Wimpenny J, Nichols W, Stickler D, Lappin-Scott H, editors. Bioline, pp 1-5
- Zadik Y, Sandler V, Bechor R, Salehrabi R. Analysis of factors related to extraction of endodontically treated teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Nov; 106(5):e31-5.

3. Materials and methods

The thesis describes the results of an observational clinical study.

The study was performed within the guidelines of the World Medical Association Helsinki Declaration of 1975 for biomedical research involving human subjects, as revised in 2000 (World Medical Association 2000).

This paper was firstly discussed in 1964 in Helsinki, Finland and has undergone to six revisions and two clarifications.

The purpose of the Declaration is to give the ethical guidelines for human medical research, and consists in 32 paragraphs. There is a certain lack of consensus worldwide about the declaration, for example the FDA recognize only the third declaration and the European Commission only the forth.

Despite these controversy, most of the research centers, as IRRCCS Istituto Ortopedico Galeazzi where the study was performed, require to fulfill the statements to begin a research project.

The observational period was divided into four clinical phases: patient recruitment, tooth extraction and implant placement, prothesis pathway, follow up.

The patient recruitment was performed following strictly inclusion and exclusion criteria.

- *Inclusion criteria.*

a) The inclusion criteria firstly involved patient with one or more teeth with a chronic periapical lesion of endodontic or periodontal origin.

b) The diagnosis is made through clinical and radiogaphic evaluation.

c) From a periodontal point of view, the extraction is decided when bone loss around the tooth cause an irreversible growing of tooth movement and the consequent impossibility to maintain it.

d) From an endodontic point of view the extraction was performed when both the orthograde re-treament and the apical surgery were judged unfeasible. Apical surgery was rejected as a treatment solution when there was a cast post in the third apical portion or the crown-root ration could be unfavorable

e) Buccal bone height loss should not be more than 4 mm

f) The age of the patient should be older than 18 years.

g) The general medical situation should referred to American Society of Anesthesiologists (ASA) class-1 or class-2.

h) The oral hygiene of each patient was examined through the Full Mouth Plaque Scores (FMPS) and Full Mouth Bleeding Scores (FMBS). Only the patients with values under 25% for both the indexes at study baseline were enrolled.

i) In the preoperative phase through careful radiographic and clinical evaluation the presence of adequate quality and quantity of native bone to achieve primary stability was evaluated and considered necessary to enroll a subject.

f) The presence of a sufficient mesio-distal space for immediate implant placement was another inclusion criteria

All the patients enrolled in the study provided written informed consent

- *Exclusion criteria*

a) The patients declaring in anamnesis any contributing medical history in which any elective oral surgical intervention would be contraindicated were excluded from the study.

b) In the same way patients with any disease, condition, or medication that might compromise healing or osseointegration should not be enrolled.

c) Untreated caries were another exclusion criteria.

This was an observational study and lasted three years; that is why patients declaring inability or unwillingness to return for follow-up visits were excluded.

As FMPS and FMBS < 25% were tacking into account as inclusion criteria, patients showing inability or unwillingness to maintain a good level of oral hygiene throughout the study were ruled out.

After diagnosis and treatment planning are formulated the inclusion and exclusion criteria are checked and the patient's data are recorded.

Before subjects may be entered into the study, the patient has to be fully informed about the study and must personally sign and date the consent form before enrolment.

All patients meeting selection criteria that give their consent to participate to the study will be consecutively enrolled.

- *PRGF preparation*

Peripheral blood will be obtained pre-surgically prior to the administration of local anesthesia. The amount of blood aspirated may range from 5 to 20 ml, depending upon the size of the gap defect to be filled. The blood is deposited in 5 cc laboratory glass tubes (blood collecting tubes®, BTI) which contain 3.8% trisodium citrate as anticoagulant. The tubes will be centrifuged at 460G, at room temperature for eight minutes in a centrifuge unit specifically designed for use with this technique (PRGF system®, Vitoria, Spain). After centrifugation, the blood will separate into distinct layers with the cellular components (mostly red blood cells and a thin layer of white blood cells) remaining at the bottom of the tube, and the plasmatic component above. The location of the red blood cell line in the calibrated centrifuge tube is dependent of the patient's hematocrit value. The plasmatic component is divided in four fractions different by mean molecular weight. In ascending order towards the top of the centrifugate, these fractions can be described as: a) plasma very rich in growth factors (PVRGF), located in a 0.2 cc layer immediately above the cellular fraction; b) plasma rich in growth factors (PRGF), located in the following 0.3 cc layer; c) plasma with growth factors (PGF), located in the subsequent 0.5 cc layer; d) plasma poor in growth factors (PPGF), located in the most superior (about 1 cc) layer (Giannobile 2002).

The 0.5 mL plasma fraction located just above the red cell fraction, but not including the buffy coat, was collected and deposited in a glass dish. In order to initiate clotting and the formation of a three-dimensional fibrin matrix for the continuous release of growth factors and proteins, PRGF activator® was added to the liquid PRGF preparation (50 mL PRGF activator® per milliliter of preparation). In order to prepare the autologous fibrin membrane, the milliliter of plasma fraction located at the top of the tubes was transferred to a glass bowl. After adding PRGF activator®, it was incubated at 37°C for 40–45 minutes, allowing the formation of a biocompatible fibrin with excellent elastic and homeostatic properties (Anitua 2009).

The total preparation time for this technique is approximately 10-15 minutes. Depending on the specific clinical circumstances (size of the defect), the application of the PRGF technique may follow two alternative protocols.

The PRGF preparation can be mixed with a grafting material (autogenous bone chips or an allograft) to enhance consistency and handling properties of the graft. About 50 microliters of 10% CaCl2 are added per each cc of PRGF concentrate to enable clot formation that will engulf graft material and create a semi-solid mass that is stable and easy to compact and manipulate at the surgical site.

When the platelet concentrate is used without a grafting material, 50 microliters of 10% CaCl2 are added per each cc of PRGF concentrate. Although stable clots develop in 5 to 8 minutes at room temperature, in both protocols maintenance of the preparation at 37°C will enable this period to be reduced to about 3 minutes.

- Sedation

In case of phobic patients beyond local anesthesia, sedation could be arranged.

Sedation is an anesthesiology technique which can be performed in private practice by using benzodiazepine or nitrous oxide (Clauser 2011).

It is a controlled medical condition and the patient loses the primary reflections, becomes relaxed, has amnesia of the surgery and the pain can be controlled.

The anesthetist must evaluate all the clinical history of the patient before the surgery and give his approval.

The procedure is solely realized on an empty stomach, needs a venous access and a preliminary control of the vital parameters. During the surgery, cardiac and respiratory frequency, arterious pressure and saturation must be monitored.

The most used drug for sedation is midazolam, given through venous access in dose of 0.07-0.08 mg/kg.

After the surgery the patient recovered in few minutes, but some parameters must be evaluated before let him go.

These parameters are:

- corporal temperature

- Arterious pressure (within the 15% of pre-sedation value)
- Cardiac frequency not less than 60 bpm
- Respiratory frequency not less to 12 breathing acts / minute
- The patient has to be waking and well oriented
- No evidence of nausea, vomit or any indisposition. (Clauser 2011)

- *Surgical procedure*

One hour before surgical procedure, patients will begin a prophylactic regimen with 2g of amoxicillin and clavulanic acid.

The removal of the tooth was performed under local anesthesia with articaine chlorhydrate 4 % and adrenaline 1:100000.

The extraction was performed carefully and less invasive as possible to preserve the socket wall and the residual bone (Figure 1).

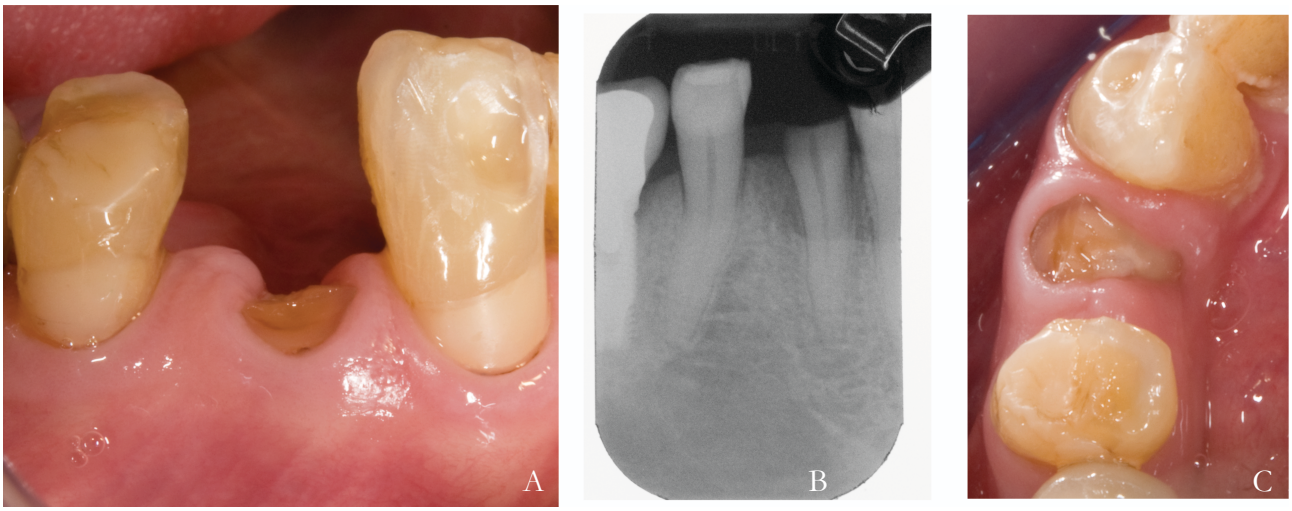


Figure 1: tooth with vertical root fracture, clinical (A, C) and radiographic (B) aspects

Full thickness mucosal flaps was raised through a scalpel with a microbevel. The tooth was carefully luxated. The extraction of the mobilized tooth was made with forceps and, thus, a minimum amount of mechanical trauma was applied to the surrounding bone. The periodontal ligament attached to the bone in the socket walls was left undisturbed. When the extraction was performed, it was necessary to clean the socket from the remaining infection. In case of endodontic lesion, the apical granuloma could remain attached to the apical portion of the root extracted, and the socket simply needed to be dried and debrided. The same for a tooth extracted due to periodontal reasons.

If the lesion was partially or not at the all removed with the apex, it was necessary an accurate debridment of the socket until the lesion and the granulation tissue was fully removed.

Implant surgical procedure was immediately performed after extraction of the involved tooth (Figure 2).

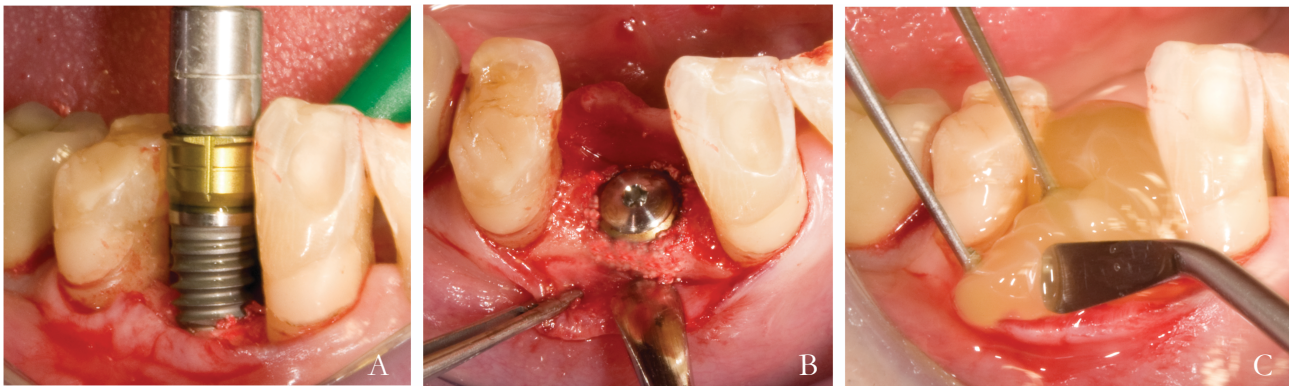


Figure 2: implant placement. A: the insertion of the fixture, embedded in liquid PRGF

B: the graft mixed with PRGF. C: a membrane of PRGF covers the implant and the graft

The apical portion of the socket was carefully prepared using pilot and twist drills and implant installation was performed according to the protocol provided by the manufacturer (BTI Biotechnology Institute; Vitoria, Alava, Spain). An implant of a proper length was installed in the fresh extraction socket, aiming at achieving primary stability, in combination with the placement of the PRGF clot (mixed or not with grafting material, following the indication of Huynh-Ba 2009). Before placement, the implants were embedded carefully in liquid PRGFs to bioactivate the implant surface. A part of the PRGF clot could also be flattened and used as a covering membrane before flap closure. If, for any reason, immediate placement of the implants was not possible, healing of the socket was allowed and implants was inserted in a subsequent surgical phase (3-5 months later).

According to the periodontal biotype a cover screw or a healing cap was attached to the implant. The flaps was replaced and secured with sutures. The flaps were repositioned and secured with nonabsorbable 5-0 silk sutures. All implants was semi-submerged so that all parts of the defects was covered by mucosal tissue.

A standardized periapical x-ray was taken at the end of surgery.

After the surgical phase, a standard pharmacologic protocol was prescribed of nimesulide 100 mg twice daily for pain control, if needed, and 0.2% chlorhexidine digluconate mouthwash (Curasept; Curaden Healthcare, Milan, Italy) twice daily for 1 week for plaque control. A soft diet was recommended and the avoidance of contact of the surgically involved zone with food for a few days, if possible.

At 1 week after surgery, the sutures were removed (Figure 3).

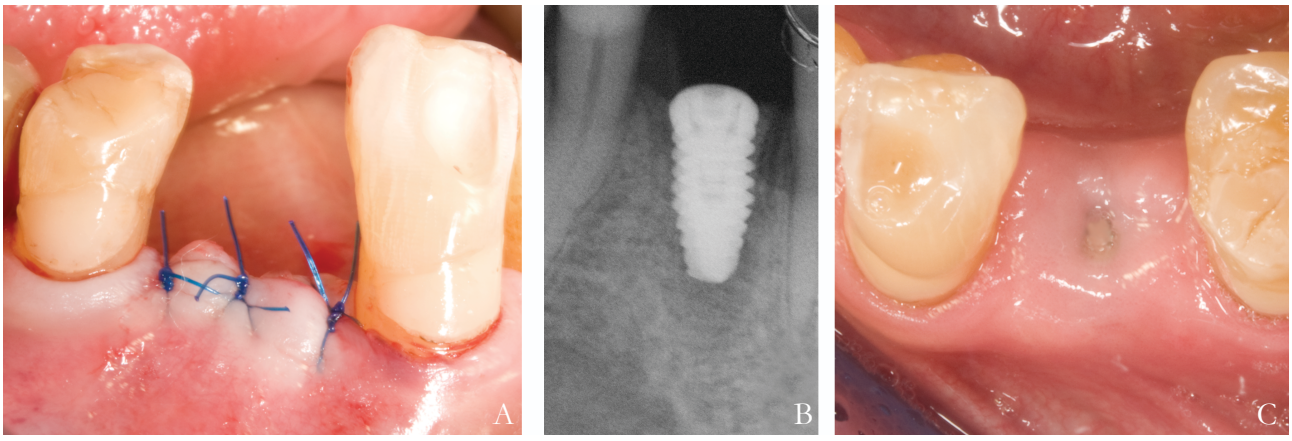


Figure 3: healing. A: the wound healing at seventh day. B: radiographic evaluation. C: healing after 14 days

After 3 to 4 months of healing, a surgical re-entry procedure was performed. Full-thickness flaps were elevated to allow access to the marginal portion of the implant sites and healing abutments of the proper height were placed. Healing abutment vary in height, width and profile: the clinician choice the proper to mould the per-implant-tissues during healing and prevent tissue overgrowth.

- *Prosthetic phase (Bhakta 2011)*

The prosthetic phase was scheduled to begin 1 month after the re-entry procedure.

It was decided to use the open tray technique to take the impression.

The open tray technique was scheduled that after removing the healing abutment, the appropriate impression coping are selected and fixed. An impression plastique tray was adapted to the dimension of the mouth and a window was opened in correspondence of the implant sites.

The open tray was tried to make sure that impression coping could emerge. This was very important for the subsequent easy removal of the impression coping.

The window was then sealed with red wax. The impression was finally taken with Polyethers impression material (eg Impregum, 3M ESPE, UK).

The material was used both in the impression tray both in an appropriate syringe which permits to take the material directly to the impression coping and obtain the more precision as possible.

After six minutes the impression copings were unscrewed through the window on the tray and the impression was removed from the mouth along with all the impression copings in place. In the end the healing abutments were replaced and color of the natural dentition of the patient was noted.

Fixed and cemented metal ceramic crown on titanium abutment was decided as rehabilitation plan for all the implants inserted.

In according with this, after the impression, the dental technician provided the provisional crown, the abutment and the metal structure of the future metal ceramic crown.

The abutments and the metal structures were tried and an x-rays was taken to verify the marginal closure of each component. If everything was right, the occlusion was taken thanks to Pattern resin on the top of the metal structure, and an impression was taken to confirm the position of the abutment.
The abutments were torqued and the provisional crowns were placed and adapted to the occlusion of the patient.
In the last appointment the metal ceramic crown was tried and finally cemented with Implacem (Implacem, DENTALICA, Milano, Italy) (Figure 4).

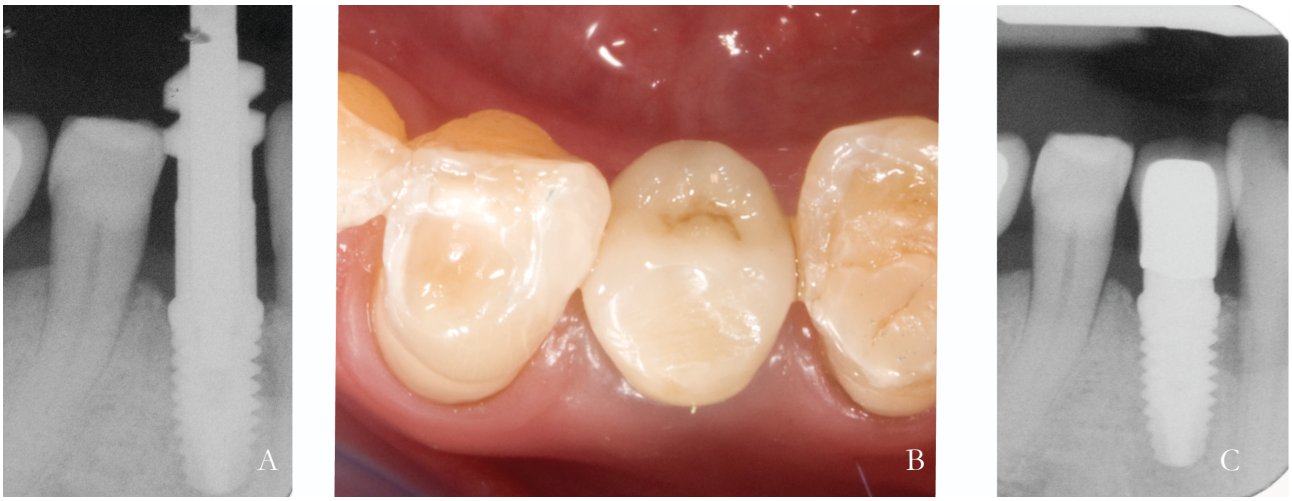


Figure 4: the prosthetic phase. A) impression coping B) final restoration, clinical view. C) final restoration, radiographic evaluation

All the prosthetic rehabilitations were made by the same dental technician.

- Radiographic evaluation

Standardized intraoral radiographs were taken at entry, immediately after surgery (baseline), at the prosthetic phase, and at each follow-up visit (after 6 and 12 months of prosthesis function). Radiographs were taken using a long-cone paralleling technique and individual trays to ensure reproducibility. Image plate are used due to reduce x-ray exposure to the patients and the very good quality of the image obtained (VistaScan Perio Plus. DÜRR DENTAL AG, Gechingen, Germany). A dedicated image analysis software (DBSWIN Imaging Software, DÜRR DENTAL AG, Gechingen, Germany) was used to perform measurements of marginal bone level around implants at the mesial and distal aspects. The implant neck was the reference for each measurement. The mesial and distal values were averaged to have a single value for each implant.

- Variables assessed

The variables assessed were implant survival, the number and type of complications, and the mesial and distal changes of marginal bone level. We also evaluated the effect of implant location, lesion type, smoking status, and bone quality according to the classification of Lekholm and Zarb (1985) on the clinical and radiographic outcomes.

Bibliography

- Anitua E, Prado R, Orive G. A Lateral Approach for Sinus Elevation Using PRGF Technology. Clin Implant Dent Relat Res. 2009 Oct;11 Suppl 1:e23-31.
- Bhakta S, Vere J, Calder I, Patel R. Impressions in implant dentistry. Br Dent J. 2011 Oct 21;211 (8):361-7
- Clauser C, Weinstein T, Capsoni F, Inquadramento diagnostico e valutazione sistemica del paziente in Manuale di Chirurgia Orale, Società Italiana di Chirurgia Orale, pp 89-124, 2011, Elsevier, Milano
- Giannobile WV. Periodontal tissue engineering by growth factors. Bone. 1996 Jul;19(1 Suppl): 23S-37S.
- Huynh-Ba G, Pjetursson BE, Sanz M, Cecchinato D, Ferrus J, Lindhe J, Lang NP. Analysis of the socket bone wall dimensions in the upper maxilla in relation to immediate implant placement. Clin Oral Implants Res. 2010 Jan;21(1):37-42.
- Lekholm U, Zarb GA Patient selection and preparation. In: Branemark P-I, Zarb GA, Alberktsson T., Tissue integrated prostheses: osseointegration in clinical dentistry. Ed Quintessence, Chicago, IL, USA, 1985, pp. 199-210.
- Weibrich G, Gnoth SH, Otto M, Reichert TE, Wagner W. Growth stimulation of human osteoblast-like cells by thrombocyte concentrates in vitro. Mund Kiefer Gesichtschir. 2002 May; 6(3):168-74.
- World Medical Association: Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 284:3043, 2000

4. Results

A total of 29 partially edentulous patients were consecutively treated and enrolled in the protocol study during the three years of his duration.

The sample was formed by 11 women and 18 men. A total of 38 implants were immediately inserted in fresh postextraction socket.

The minimum follow up had to be 1 year after the delivered of definitive prostheses.. Due to this limit 9 patient had to be excluded and the final sample was constituted by 6 women and 14 men. The mean age at surgery was 51.7+-12.6 (SD), range between 29 and 71. The teeth were extracted due to periodontal lesion (14), endodontic lesion (9) and the remaining for vertical root fracture (6) (Figure 1).

The implants finally analysed were 29. Nine of these were inserted in the mandible while the remaining 20 were inserted in maxilla. A total of 6 partial prostheses and 14 single tooth restoration were delivered and loaded according to a delayed protocol, 4 months after surgery. The implant distribution is shown in figure 2. The premolar region was the mostly treated in maxilla, while first molar was the most extracted tooth in mandible (Figure 3 and 4).

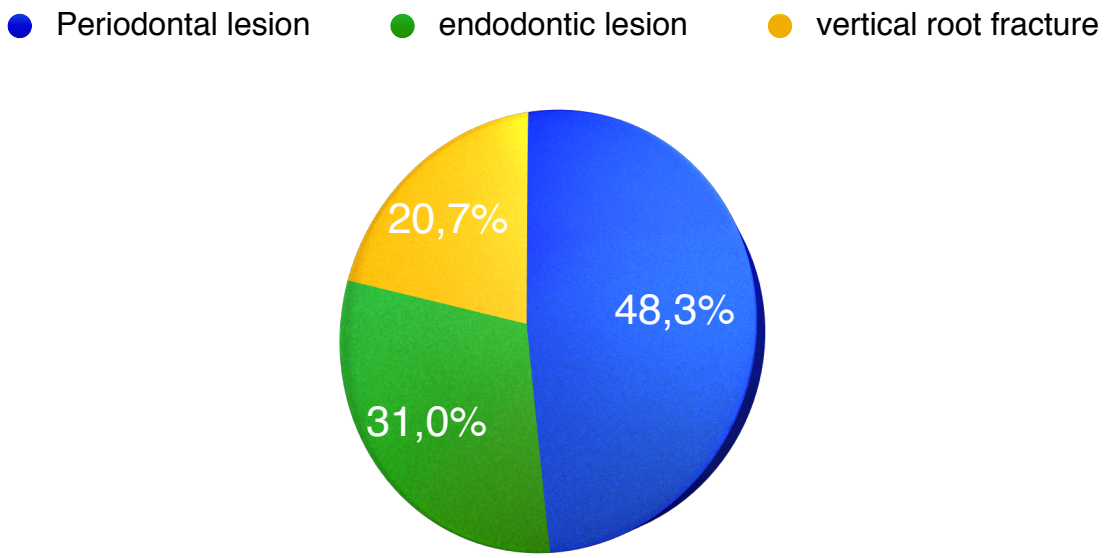
From a medical point of view, all patients were classified as ASA-1, except two ASA-2. The smoking habit was evaluated both in quantity (number of smokers) and quality (number of cigarettes/die).

The total number of smokers was 6; one of them smoked more than 15 cigarettes, the remaining 5 smoked less than 10 cigarettes per die. The "heavy smoker" had to extract all his remaining teeth due to periodontal lesions, the remaining 5 due to endodontic lesion.

The implant survival rate was 100% after 1 year of function. No failure of the fixture was registered. All prostheses were successful and functioning.

At 1 year of function the patients were re-called for a clinical and radiographic evaluation.

All the patients responded. The peri-implant bone loss was evaluated through periapical radiograph. The results was 0.38 ± 0.14 mm. This value was not affected by implant location, smoking status, lesion type, or bone quality.



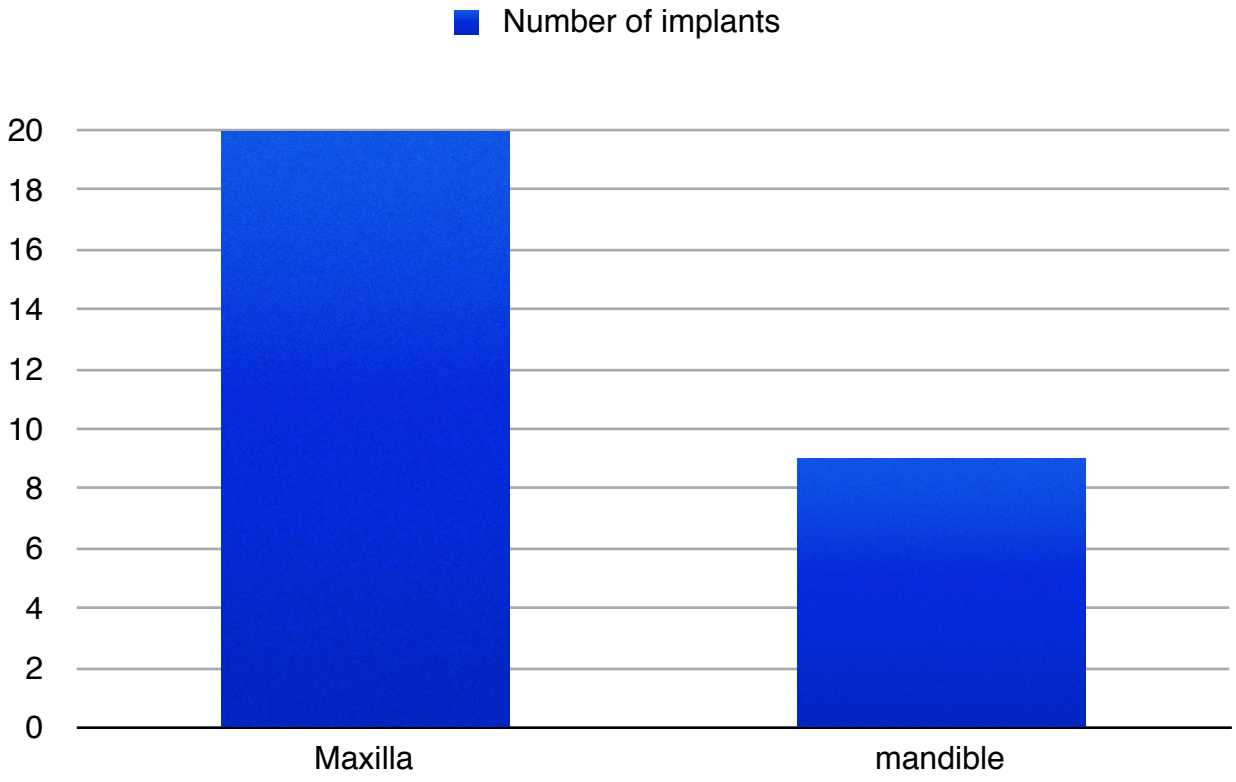
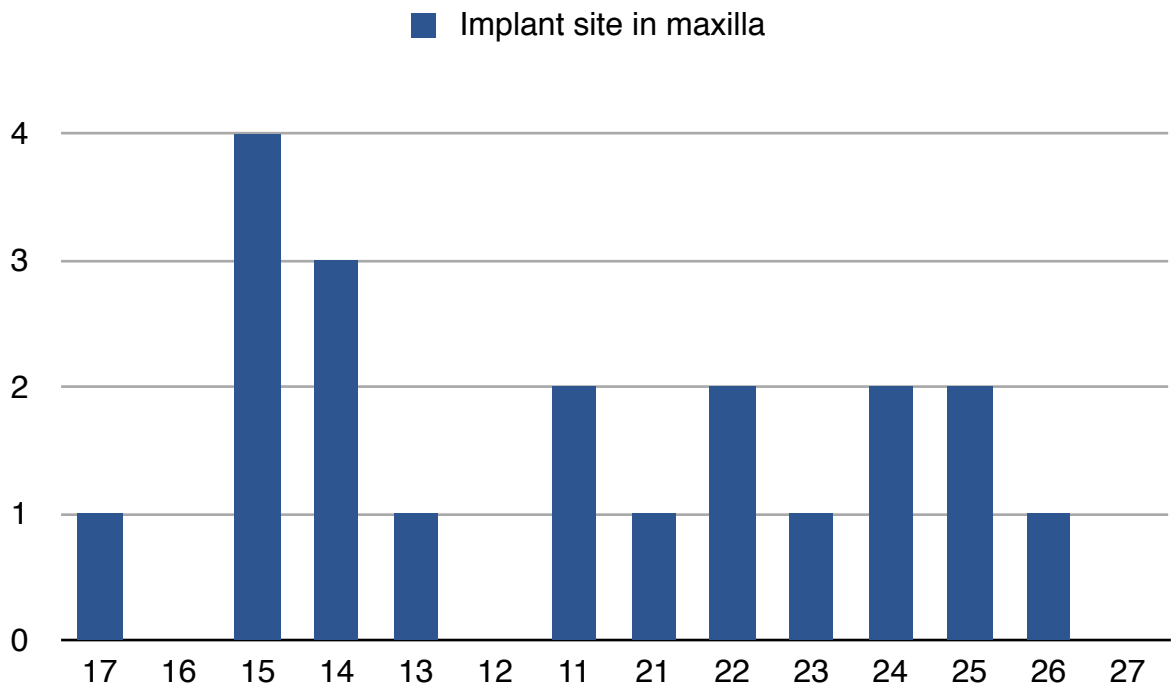


Figure 1: On the causes of the extraction of the teeth in maxilla and mandible

Figure 2: distribution of implants inserted



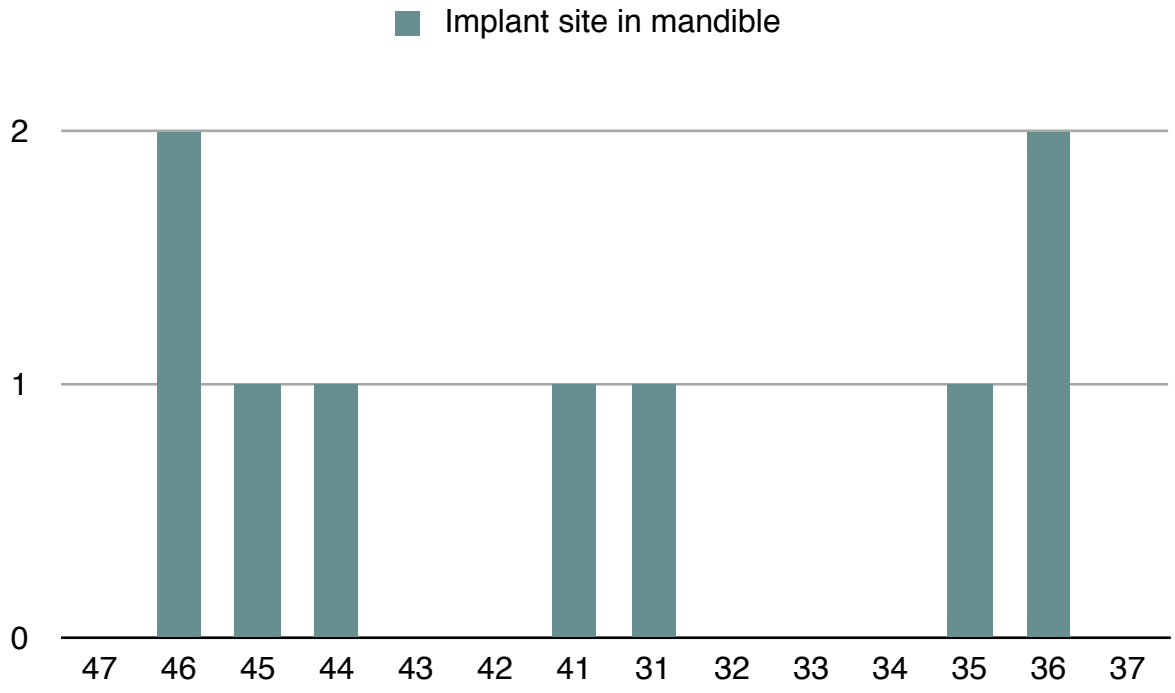


Figure 3 and 4: detail of the site where implants were placed

5. DISCUSSION

5.1 Implants in infected site: what was the reason of infection?

In this study was noted the reason why each teeth had to be extracted.

The statistics performed revealed they were extracted due to periodontal lesion (14), endodontic lesion (9) and vertical root fracture (6).

• *Endodontic disease*

Endodontic disease concerns all the pathologies located in the radicular and periradicular area.

It was stated out that microbial agents play an essential role in the progression and perpetuation of disease.

Apical periodontitis is inflammation and destruction of periradicular tissues caused by etiological agents of endodontic origin (Nair 2004).

The tooth pulp becomes infected and then necrotic by autogenous bacterial microflora. The flora is predominantly anaerobic and the environment of the radicular canal provides the habitat to his sustainability.

This permits the establishment of a polymicrobial community which has the following pathogenic properties:

- antigenicity,
- mitogenic activity,
- chemotaxis,
- enzymatic histolysis,
- activation of host cells.

How bacteria reach the pulp chamber

Kakehashi in 1965 demonstrated that apical periodontitis could not develop without the presence of microbial flora int he oral cavity. He carried out his studies in rats.

Moeller in 1966 improved this findings determining the essential role of obligate anaerobes in endodontic infections.

Hence the research had to focus how the bacterial complex could reach the pulp tissue.

It is necessary an opening in the dental hard tissue wall which can been affected from caries, clinical procedures or trauma induced fractures and cracks.

In some cases, necrotic pulps could occur in intact crowns: the reasons is probably the presence microcracks not detectable.

Grossman in 1967 also suggested the possibility for periodontal bacteria to reach the pulp chamber through severe periodontal blood vessels.

The cervical root surface could also been penetrated from bacteria, through exposed dentinal tubules, due to gaps in the cement coating.

Therefore, exposure of the dental pulp to the oral cavity is the most important route of endodontic infection (Nair 2004).

How investigate the bacterial flora of endodontic infection

In the past, microorganisms were identified on the basis of the morphology by cell cultivation-based technique (Siqueira & Rôcas 2005). It consists in bacterial growth under specific set conditions at the presence of high-nutrient artificial growth media (Hugenholtz 2002). The most notably difficulty is to simulate the proper environmental conditions required for microorganisms to grow. Therefore some taxa having specific nutrients and growth conditions requirements remain uncultivable. Furthermore other bacteria are difficult to cultivate because they enter in a quiescent- uncultivable state in response to adverse conditions (Spratt 2004). The uncultivable bacteria issue may underestimate the bacterial species variability therefore the detected easily grown microorganisms may not necessary represent the dominant or clinically significant species of that environment/sample. In addition, traditional method has low sensitivity, depend on the investigator's experience and it is time-consuming.

With the progress in the field of molecular biology, the molecular techniques replaced the traditional culture method by identifying the microorganism through the microbial genome analysis (Tang et al. 1997). The principle behind the molecular approach is to study the microbial diversity by targeting specific DNA sequences which are indicative of microorganism species. Conserved DNA regions are identical in all members of a given domain (Bacteria, Archaea, Eucarya) while variable DNA regions contain unique information about genus and species of the microorganisms. 16 rRNA- encoding genes is targeted to identify bacteria and archaea while 18S rDNA for fungi identification (Siqueira & Rôcas 2005). The high sensitivity and specificity of molecular techniques permit to identify a broader range of bacterial species even the uncultivable bacteria giving a more precise and realistic description of the contaminating-bacteria. Furthermore a large number of samples can be rapidly processed shortening the processing time. On the other hand, all the detected species may not be involved in the aetiology or may not have any clinical importance. In addition, the molecular techniques by detecting microorganisms DNAs do not distinguish between viable and non viable cells, between extracellular microorganisms in the periapical tissues and phagocytosed microorganisms (Nair 2004). Polymerase chain reaction technique (PCR), DNA/DNA hybridization, Fluorescence In Situ Hybridization (FISH) are the most commonly techniques used and herein they have been considered and summarized briefly.

- Polymerase chain reaction technique

PCR technique is a target DNA's amplification process where through repetitive cycles of double strand DNA denaturation, primer annealing and extension, exponential number of copies are synthesized (Mullis et al. 1994, Mullis 1990). After amplification, DNA is sequenced and compared with all the sequences present on the databases (Blast or RDP Ribosomial Database Project) to find its

homology sequence on the base of the closest match and thus is identified. There are multiple variations of PCR procedure: nested PCR, real time PCR, Reverse Transcriptase- PCR, multiplex PCR (Siqueira & Rôcas 2005; Siqueira & Rôcas 2003; Tang et al. 1997). However it is always targeted short bacterial DNA sequence (16S rDNA) containing unique information essential for microorganism identification. Extremely high sensitivity (1-10 cells can be detected compared to 10^4- 10^5 cells needed for culture methods and 10^2-10^4 cells needed for DNA/DNA hybridization), accuracy and process rapidity are the main advantages.

By the contrast, in addition to the previously mentioned limitations of molecular genetics techniques, some bacterial species show very close relationship so the gene sequence information do not allow to easily and surely distinguish between these taxa. For example among the endodontic infective bacteria, this is the case for Streptococci (S. mitis, S. oralis, S. sanguinis, S. gordonii), Actinomyces app (A. naeslundii, A. israelii, A. meyeri, A. odontolyticus, A. viscosus, A. gerencseriae, A. radicidentis), coagulase-negative Staphylococci (S. epidermidis, S. warneri, S. lentus) and Veillionella spp (V. parvula, V. atypical, V. dispar) (Spratt 2004).

- DNA/DNA hybridization

Checkerboard DNA/DNA hybridization is a procedure that employs DNA probes to target the whole genomic DNA or specific genes (16S). A large number of DNA samples are hybridized against large number of DNA probes on a single support membrane, allowing detection of multiple DNAs in a single or multiple samples (Socransky et al. 1994). DNA probes show cross-reactivity: may hybridize with closely related microorganisms DNAs giving false results; it provides information regarding only the target expected microorganisms thus the unexpected bacteria are not detected. When the whole genomic probe is used, bacteria need to be initially cultured, so only the cultivable bacterial species can be detected. Another drawback is the detection limit: it is needed a sufficient number of cells (10^3- 10^4) in order to be detected.

- Fluorescence In Situ Hybridization (FISH)

FISH is a hybridization method based on the complementary binding between fluorescent labelled probe and the target16S rRNA. FISH technique gives information regarding quantity, morphology, spatial distribution of found micro-organisms (Moter & Gobel 2000; Siqueira & Rôcas 2005). The accuracy and reliability depends on the chosen probes. Low signal intensity can be caused by insufficient probe penetration into bacterial cell (Moter & Gobel 2000).

The histological analysis of tissue section of root apex together with the surrounding pathological tissue provides information for understanding the establishment and progress of the extraradicular infection. Once the tissue biopsy is stained, presence and spatial localization of bacteria, biofilm structure, presence and distribution of acute and chronic inflammatory cells can be visualized at the light microscope. Modified Brown and Brenn staining technique is commonly used to visualize Gram negative and Gram positive bacteria, Actinomyces and additional tissue elements (Ricucci & Siqueria 2010; Ricucci & Siqueria 2009; Ricucci & Siqueria 2008; Nair 2006).

How endodontic flora express is pathogenicty

Individual species of endodontic flora usually have low virulence. They become pathogenic through a combination of factors which can be summarise in four points (Nair 2004):

- Interactions with other micro-organisms in the root canal, to develop synergistically beneficial partners;
- the ability to interfere with and evade host defenses;
- the release of lipopolysaccharides (LPS) and other bacterial modulins;
- the synthesis of enzymes that damage host tissues

How the host respond

Apical periodontitis is viewed as the consequence of a dynamic encounter between root canal microbes and host defense (Nair 2002.)

The majority of the blood cells involved in host response to apical infections are polymorphonuclear leukocytes (PMN), lymphocytes, plasma cells, and monocyte/macrophages. Structural cells include fibroblasts, osteoblasts, and epithelial rests.

Stashenko in 1995 focused the mainly importance of PMN and monocyte.

How the apical periodontis develops itself

The interaction between bacterial pathogenic flora and host response results in apical periodontis, which can appears in various form as summarised in figure 1.

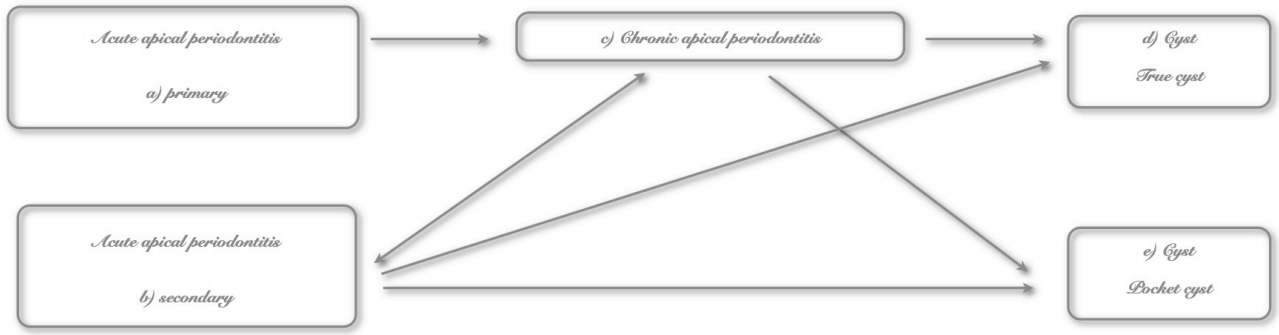


Figure 1: apical periodontitis and its development

(from Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures.. Crit Rev Oral Biol Med. 2004 Nov 1;15(6):348-81).

The *initial apical periodontitis* is generally caused by micro-organisms residing in or invading from the apical root canal into the periapical tissues. Also accidental trauma, injury from instrumentation, or irritation from chemicals and endodontic materials can cause it.

Typical symptoms are pain, tooth elevation, and tenderness to pressure on the tooth. The tissue response is generally limited to the apical periodontal ligament and the neighboring spongiosa. It is initiated by the typical neuro-vascular response of inflammation, resulting in hyperemia, vascular

congestion, edema of the periodontal ligament, and extravasation of neutrophils.In a few days' time, the bone surrounding the periapex can be resorbed due to the intervention of osteoclasts, and a radiolucent area may become detectable at the periapex (Stashenko 1992).

The self-induced destruction of the tissues prevents the spread of infection to other parts of the body and provides space for the infiltration of specialised defense cells.

Cytokines intensify the local vascular response, osteoclastic bone resorption, and effector-mediated degradation of the extracellular matrices and can place the body on a 'general alert' by endocrine action, to raise the output of acute-phase.

Acute primary apical periodontitis has several possible outcomes, such as: spontaneous healing, further intensification and spreading into the bone (alveolar abscess), open to the exterior (fistulation or sinus tract formation), or becoming chronic.

Chronic apical periodontitis is commonly referred to as 'solid dental' or 'periapical granuloma'. It consists of a granulomatous tissue with infiltrate cells, fibroblasts, and a well-developed fibrous capsule (Nair 1996). The connective tissue capsule of the lesion consists of dense collagenous fibers that are firmly attached to the root surface, so that the lesion may be removed in toto with the extracted tooth.

Periapical cysts are a direct sequel to chronic apical periodontitis, but not every chronic lesion develops into a cyst (Nair 2004).

There are two kind of radicular cysts: 1) cysts containing cavities completely enclosed in epithelial lining, 2) cysts containing epithelium-lined cavities that are open to the root canals (Simon 1980, Nair 1996).

Periapical true cyst develops itself in three stages. During the first phase, the dormant epithelial cell rests (Malassez, 1884, 1885) are believed to proliferate, probably under the influence of growth factors (Thesleff, 1987; Gao et al., 1996; Lin et al., 1996) that are released by various cells residing in the lesion. During the second phase, an epithelium- lined cavity comes into existence. During the third phase, the cyst grows, but the exact mechanism has not yet been adequately clarified.

The periapical pocket cyst is probably initiated by the accumulation of neutrophils around the apical foramen in response to the bacterial presence in the apical root canal (Nair 1996; Nair 1997).

The micro- abscess so formed can become enclosed by the proliferating epithelium, which, on coming into contact with the root-tip, forms an epithelial collar with 'epithelial attachment' (Nair and Schroeder, 1985). When the externalized neutrophils die and disintegrate, the space they occupied becomes a microcystic sac. Bone resorption and degradation of the matrices, occurring in association with the enlargement of the pocket cyst, are likely to follow a similar molecular pathway, as in the case of the true periapical cyst (Nair 1996). In the end, this lesion has some structural and pathological issue in common with periodontal pocket, justifying the name periapical pocket cyst (Nair 1996).

Secondary endodontic lesion

When the microorganisms are not eliminated by the endodontic treatment, their persistence presence causes the apical periodontitis to be recurrent leading to the formation of a secondary periapical lesion (Nair 2006, Haapasalo 2011)

The post-treatment endodontic disease is defined as "the presence of a periradicular inflammatory lesion (apical or lateral) in an endodontically treated tooth when the lesion is no longer can be assumed to be undergoing healing after the root canal treatment" (Haapasalo 2011). At a radiographic examination the inflammation and the bone destruction are seen as radiolucencies around the tooth apex.

The aetiology and treatment of secondary periapical lesions are more complicated (Nair 2006) than the primary lesions. The frequency of the secondary periapical lesions ranges between 16 and 65 % depending on the published articles (Soikkonen 1995; Sidaravicius et al. 1999; De Moor et al. 2000; Kirkevang 2001; Dugas 2003).

A study published by Siqueira and Rocas showed that 158 bacterial and 3 fungal species are involved in the aetiology of the secondary apical infections (Siqueira & Rôças 2009). Cultural studies showed that one of the most common bacterial species found in this type of lesions is Enterococcus faecalis (Molander et al. 1998; Pinheiro et al. 2003; Sundqvist et al. 1998).

Bacteria can also be found outside the tooth canal system: they can form a biofilm- like structure at the root surface (Tronstad et al. 1990) or cohesive colonies within the inflamed lesion (Figdor 2002) which is usually symptomless.

In spite of the endodontic treatment, the persistent apical periodontitis is considered to be mainly caused by those extra-radicular microorganisms (Tronstad et al. 1990, Figdor 2002, Byström et al. 1987).

In the majority of the cases, primary and secondary apical lesions are caused by bacteria but the bacteria composition may differ. However tooth filling-material extravasation, root vertical fracture and cholesterol crystals can be other possible causes (Haapasalo et al. 2011).

Secondary lesions are caused by those bacteria species which were not eradicated by the primary endodontic treatment or by those which penetrated the extraradicular area during the endodontic treatment. Therefore they are resistant bacteria able to grow in a rigid environment with less nutrient supply (Sakamoto et al. 2008).

In the intraradicular canals of persistent apical periodontitis tooth, Gram- Positive bacteria can be found (Möller 1966; Molander et al. 1998; Sundqvist et al. 1998), and in the majority of the studies, the most frequently detected Gram- Positive bacterium is E. Faecalis (Möller 1966; Molander et al. 1998; Hancock et al. 2001; Peciuliene et al. 2000; Peciuliene et al. 2001; Sundqvist et al. 1998; Pinheiro et al. 2003; Siqueira & Rocas 2004; Engström 1964; Zoletti et al. 2006;Sedgley et al. 2006;Gomes et al. 2008). In the primary lesions the detection of E. faecalis is a rare event and it is considered a transient oral bacterium (Aas et al. 2005).

A recent study compared the E. faecalis strains isolated from devitalized root canals and those isolated from the intestinal tracts to study whether E. faecalis from endodontically treated teeth has an endodontic origin (Vidana et al. 2011). The study concluded that intestinal-isolated strain and devitalized tooth-isolated strain have a completely different genetic origin. Consequently E. faecalis found in endodontically treated teeth with secondary lesions has an exogenous origin: the bacterium is brought to the root canals during the primary ortograde treatment or at the end of the treatment or through ingestion of infected food (Rôcas 2004; Boyce 1994; Foulquie-Moreno 2006).

Other intraradicular bacterial species which may also cause the secondary periapical lesions are: Streptococcus, Candida (especially C. Albicans) and Propionibacterium (Figdor & Gulabivala 2011).

Understanding the aetiology of periradicular lesions is complicated because bacteria may also infect the extraradicular area (Nair 2004; Sundqvist1980; Haapasalo 1987; 67. Tronstad 1987; Sunde 2002; Sunde 2003; Sunde 2000; Nair 1998c). This possibility is confirmed by the fact that when an abscess or fistula is present, which are clinical symptoms of secondary periapical lesions, bacteria are found in the extraradicular area (Haapasalo 2003; van Winkelhoff 1988; Trope. 1992). Few bacterial species are able to survive outside the root canals because humoral and cellular-mediated immune response is activated in order to eradicate the infection which is spreading in the extraradicular area. Therefore microorganisms contaminating the extraradicular area are those able to survive in this hostile environment. For example, A. israelii and A. radicidentis elude the host immune system response by avoiding the phagocytic mechanism through bacterial aggregation (Figdor & Gulabivala 2011).

Persistent apical lesions may be also caused by Actinomyces. In a clinical case from Ricucci, secondary persistent periapical lesion was associated with presence of a fistula and it was solved by an apical surgery. The removed root apex histological analysis showed presence of Actynomices (Ricucci & Siqueira 2008). Actinomycosis can only be diagnosed by an histological analysis since it is not associated with specific and clear clinical signs and symptoms. Ricucci and Siqueria affirm that there is not a univocal correlation between the presence of swelling, fistula and/or symptoms with actinomycosis because these clinical signs can be also caused by other different etiologic agents. They also say it is difficult to distinguish between apical periodontitis caused by extraradicular microorganisms and that caused by intraradicular microorganisms based on clinical signs and radiographic examinations. As the matter of the fact, the apical actinomycosis can only be diagnosed through an histological analysis of the lesion containing the root apical portion which is performed at the end of the apical surgery (Ricucci and Siqueria 2008).

In the literature Nair, describing the periapical disease caused by extraradicular actinomyces, says that excessive bone rarefaction, abscess, fistula and suppuration are considered as the clinical- radiographical evidences. These evidences may be indicative for the diagnosis but this will be confirmed only by an histological analysis of the lesion will confirm the diagnosis, supporting the diagnostic theory of Ricucci and Siqueria, previously mentioned. Actinomycosis is a chronic, granulomatous infectious disease characterized by suppuration, abscess formation and draining sinus tracts. Nevertheless there is

not a strict correlation between the persistence of exudation and sinus tract and the apical actinomycosis but it is the most probable event.

Those lesions caused by actinomycosis because it is an extraradicular infection,

are resistant to the host immune system response, to the action of antibiotics and also to the ortograde treatment since it is not able to reach the extradicular bacteria failing to eliminate the infection (Su 2010). The apical surgery is the only valid alternative to solve the apical periodontitis.

It has been noticed a correlation between the success of the apical surgery and the pre-operative clinical signs: the success probability in those cases having clinical evidences as fistula and pain, was inferior than those cases showing no pre-operative clinical signs (Von Arx 2010). It is believed that these pre-operative clinical signs as those just mentioned, are associated with the acute phase of infection rather than the chronic phase and thus this can prevent the healing process to be achieved (Von Arx 2010). In fact, during the acute phase of infection the immune cells as neutrophils, play a crucial role in fighting the microorganisms but at the same time they cause damage to the surrounding tissue by releasing chemical mediators. For example, bone resorption around the root apex and extracellular matrix degradation can occur. This phase ends with bacteria confined within the canals. When bacteria are not completely eliminated, their persistent presence cause the shift from the acute to the chronic phase which is characterized by a complete different set of immune cells as T lymphocytes and macrophages. This change results in a reduction of bone resorption and reconstruction of connective tissue along with no symptoms. This balance is lost when the microorganisms are able to get outside the canals reaching and colonising the extraradicular area (Nair 2004).

Nair affirms that these clinical signs and symptoms are associated with the extraradicular infection especially actinomycosis (Nair 2006). Therefore it can be deduced that some procedures adopted during the surgery for root apex removal (such as incomplete removal of the lesion or removal of the apex) do not ensure the complete eradication of extraradicular bacteria leading to the high risk of infection recurrence (Von Arx 2010). Hence this should chew on which are the most suitable procedures to be executed during the root apex removal.

Extraradicular infections: an overview

According to recent studies, persistent apical periodontitis may be also induced by bacteria colonising the extraradicular area particularly invading the inflamed periapical tissue (Tronstad et al. 1987, Gatti et al. 2000, Sunde et al. 2002, Siqueria 2003) and therefore an extraradicular infection develops. These extraradicular bacteria can form biofilms at the root surface or a well-organized actinomycotic colonies within the inflamed lesion. Consequently, the healing process cannot be achieved (Nair 2006, Happonen 1986, Sjögren et al. 1988). Furthermore, these have the ability to survive in the inflamed tissue, scavenge nutrients and evade the host immune defence.

By the contrast the intra-radicular invading- bacteria are opportunistic pathogens (Siqueira 2002) and they are not able to survive in the extraradicular area.

Microorganism pathogenicity causing the extraradicular infection can be independent or dependent on the intraradicular bacteria; for example, in the case of a dependent infection, once the intra radicular infection has been properly solved by the endodontic treatment, the extra radicular infection decreases its pathogenicity, becoming less severe. The acute periapical abscess is the most common type of extra radicular infection dependent on those intra radicular bacteria (Siqueria 2002).

When the extraradicular infection is independent, the microorganisms persist even after a successful treatment is performed. In this case the main involved species are:

Actinomyces and Propionibacterium propionicum thus causing the periapical actinomycosis. In periapical actinomycosis disease, the bacteria invading the periapical tissue establish an equilibrium with the host immune response: thanks to bacterial strategies the host response is not able to eliminate the microorganisms meanwhile the host immune cells confine the microbial colonies inside the inflamed tissue thus actinomycotic colonies become persistent leading to a chronic inflammation (Siqueria 2003).

Advanced molecular techniques, scanning electron microscopy (SEM) and cultural analysis of pathological periradicular tissues, provided evidences confirming that bacterial contamination at the surrounding tissue of a chronic apical periodontitis tooth is a common event (Block et al. 1976).

Ricucci affirms that there is a strict correlation between the microorganisms colonizing the extraradicular region and those infecting the canal system: the extraradicular infection is necessarily caused by the intradicular infection; moreover the pathogenicity of the extra is affected by the intra and vice versa. It is still unclear which one has the main pathogenic responsability: whether the extraradicular infection is the result of those microorganisms residing at the extra radicular portion playing a primary role in establishing the infection or it is the consequence of the spread of intraradicular non-solved infection beyond the root apexes (Ricucci & Siqueira 2008, Ricucci et al. 2009.)

Vertical root fracture

In this study 6 teeth were extracted due to a vertical root fracture.

Vertical root fracture (VRF) is a complication of endodontic treatment and often leads to extraction of the tooth involved.

The term 'true' vertical root fracture is defined as a complete or incomplete fracture initiated from the root at any level, usually directed facio-lingually (Rivera 2009).

The fracture may involve one proximal surface (facial or lingual) or both facial and lingual proximal surfaces. The fracture is located in the root portion of the tooth only and may extend coronally toward the cervical periodontal attachment. (Rivera 2009).

All fractures extended from the canal to at least one root surface but not necessarily to both . Usually, fractures extended to facial and lingual surfaces. Similarly, fractures often extended only the partial length of the root, usually to the apex but not always to the cervix (Rivera 2009).

Filling procedures or stress factors from mastication at the end of endodontic treatment can cause it (Endodontics: Colleagues for excellence.1997, Rundquist 2006).

Vertical root fracture is a consequence of wedging forces within the canal. These excessive forces exceed the binding strength of root dentin, causing fatigue and fracture (Rivera 2009).

When the VRF occurs, it reaches the periodontal ligament, and consequently soft tissue interpose and increase the separation of the root segment. Through gingival sulcus material, food debris, and bacteria obtain access to the fracture area: an inflammatory process is induced in the adjacent periodontal tissue (Walton 1984), resulting in periodontal ligament breakdown, alveolar bone loss, and granulation tissue formation (Bergenholtz 2003).

Where the buccal plate is thin the breakdown is especially rapid. The most susceptible teeth are maxillary premolars and the mesial roots of the mandibular molars. (Tamse 1999, Cohen 2003)

Lustig (Lustig 2000) described the pattern of resorption as dehiscence and was found in the buccal plate in 90% of the cases examined. A narrow bone cleft develops and resorbs in an apico-coronal direction; i.e., it propagates with the fracture to form an oval or oblong type of bone resorption (Walton 2002).

At the end the defect extends to the interproximal areas in mesio-distal direction.

The lingual aspects is not involved and consequently in a periapical radiograph no radiolucent area can be detected.

The prevalence of vrf is not well established. The literature indicates the prevalence is between 2% and 5% (Tamse 2006). However the diagnosis of this pathology is not easy and can be mismatched with other diagnosis, such as classic endodontic failure or progressive periodontal disease.

The more frequent sign and symptoms associated with vrf are:

- Osseous defect
- Mild pain
- Sinus tract
- Exacerbation of a chronic lesion

Osseous defect and sinus tract are typical features of periodontal disease.

Despite of this when sinus tract is present both at buccal and lingual aspects is almost pathognomonic of vrf.

The bone defect is also different in vrf. Especially at the beginning the probing area was then limited to the site that faced the fracture line in the root, which initially is narrow and difficult to locate and probe. Furthermore in a patient with no signs of periodontal disease, a deep probing pocket depth of only on site could indicate a vertical root fracture.

Nevertheless, the definitive diagnosis of vrf can be made with an exploratory flap (Meister F, 1980) (Pitts DL, 1983.) (Lin LM, 1982).

As anticipated before is very difficult to detect vrf through periapical radiograph.

The presence of a hair-like fracture line radiolucency in the dentin body is a pathognomonic sign, even if is not easy to detect it and usually not seen in routine orthoradial periapical radiographs. Nevertheless a study identify this sign in 35.7% of 375 VRF cases (Rud, 1970).

Another typical sign is the appearance of root segment separation and consequently the large bone losses surrounding the tooth or root.

There are two main causes of vrf: 1) post-placement (cementation) and 2) condensation during root canal filling

(Pitts 1983a, Pitts 1983, Ross 1991, Harvey 1981, Meister 1980, Obermayr 1991, Tamse 1988, Tamse 2006)

Condensation, both lateral and vertical, may cause excessive wedging forces, creating a vertical root fracture (Rivera 2009) while two aspects of posts cause wedging forces: wedging occurs during cementation of posts and also during the seating of tapered posts or with posts that depend on frictional retention.

- Periodontal disease

The periodontal disease are divided in three categories: chronic periodontis, aggressive periodontitis, necrotizing periodontitis.

In our study, all the teeth were lost due to periodontal disease were affected to chronic periodontitis (CP).

CP derives from plaque induced gingivitis. This kind of gingivitis is caused by the presence of bacteria on the gingival margin.

The clinical aspects of CP are:

•changing in colour, tickness and volume of gingiva marginale,

•Bleeding on probing

•Formation of periodontal socket,

•Losing of clinical attach level

•Recession of gingival margin

•Lost of alveolar bone

•Probing of furcation

•Dental mobility

•Moving and dental exfoliation

The CP is predominantly an adult infection, but could develops itself also in child.

Not every patient affected by plaque gingivitis become affected also by CP. There is a predisposition to CP.

Trombelli et al observed different answers from subject with "sperimental periodontitis" (Trombelli 2004, 2005).

The different answers could derive from different predisposition to gingivitis, mainly gentically determined (Shapira 2005 - Scapoli 2005).

The CP is the most diffused form of periodontal disease. The pathology destruction of periodontal tissue is not uniform: some teeth could be highly involved while others for none.

The progression of CP is slow and continued over the years, with occasionally moments of aggravation.

There are some risk factor affecting the prognosis and the developing of chronic periodontitis.

Bacterial plaque is the first one. The pathology is correlated to specific microflora organised in biofilm even if this bacterial aggregations could not induce the pathology alone.

The pathology is induced by these bacteria in predisposed host with cumulative risk factors.

Age is another risk factor due to the cumulative effects of the pathology during years. This explains why the prevalence of the CP increases with age.

Smoking is certified as a risk factor for CP by many trasversal and longitudinal studies (Kinane and Chestnutt. 2000); it increases the risk to develop the CP of a range between 2.5 to 7 times..

Also the response of the patient to periodontal therapy is negatively influenced by smoking. Smoking reduces the inflammatory response of the host so the clinical manifestation of the CP is not clearly as in a non smoker patient.

Systemic disease could be a risk factors for CP, even if their direct correlation is not well established due to poor evidence published study.

Wilton in 1988 assessed that a quantitative and functionally reduction of polimorfonucleate leucocyte means an augmentation of gravity and velocity of periodontal disease .

Fenitoina, nifedipina and ciclosporine could interfere with the normal answer of the gingiva to plaque (Ellis et al. 1999).

Every affection or therapy which decrease the immunity response (as HIV), could be considered a risk factor for CP (Barr 1992).

Patients with diabetes presents more prevalence and severe periodontis than patient without diabetes.

Genetic factors play an important role for the predisposition of the pathology.

Studies on twins produced great level of evidence of this association. Probably a lot of genes are involved, varying between individuals and races. Although there is a genetic predisposition for CP, gingivitis has not, and is considered as a common respond to plaque.

Microbial consideration on periodontal disease

It has been estimated that in supragingival plaque there can be 10^9 bacterial species, while in a healthy pocket 10^3 and in pathology pocket 10^8.

All these million, billion, of bacteria does not necessary lead to the lost of periodontal tissue.

There are a former equilibrium between these species, but this equilibrium can be broken by pathogenous which can cause periodontal disease.

Despite of this, the presence of pathogenous species alone is not enough to start the periodontal disease, as for other infective disease, for example lung infections which may be caused by a wide range of species.

Although periodontal disease have certain features in common with other infection disease, it presents some reason of uniqueness, and the major is the unusual anatomic feature that a mineralised structure, the tooth, passes through the integument, so that part of it is exposed to the external environment while part is within the connective tissue (Socransky 2008).

The results is that microorganisms colonize a relatively stable surface (the tooth) and are in the proximity to soft tissue (periodontium).

The organisms that cause periodontal disease are organised in biofilm located on tooth or epithelial surface.

The World Workshop in Periodontology (Consensus Report 1996) retrieved Actinomyces actinomycetemcomitans, Porphyromonas gingivalis and Treponema forsythia as principal periodontal pathogens.

Furthermore in any given plaque sample, 30 or more bacterial species are commonly detected; thus the biofilm that colonise the tooth surface is on the most peculiar.

Socransky et al (1998), through the examination of over 13.000 subgingival plaque samples from 185 adult subject, demonstrated the presence of specific microbial groups within dental plaque. Six groups were recognised and divided in colour.

The red cluster consisted of P. gingivalis. B. forsythus and T. denticola. The orange cluster consisted of F. nucleatum subspecies, P. intermedia and P. nigrescens, Peptostreptoeoccus micros and Campylobacter rectus, Campvlobacter showae, Campylobacter gracilis, E. noddttim and S. eonstetlatus. The 3 Capnocytophaga species. Catnpylobacter conclsus. Eikenella corrodens and Actinobacillus actinomycetetncomitans serotype a formed the green cluster. while a group of streptococci made up the yellow cluster. Streptococcus mitis, Streptococcus sanguis and Streptococ- cus oralis were most closely related within this group. Actinotnvces odontolyticus and Veillonella parvula formed the purple cluster. Actinotnyces naeslundii genospecies 2 (Actinomyces vLseosus), Selenomonas noxia and A.aetinomycetemcotnitans serotype b did not cluster with other species (Socransky, 1998).

The red complex is composed by species to be the major etiologic agents of periodontal disease.

As stated before, microrganisms alone cold not lead to periodontal disease alone.

The habitat has a major effect on the composition, metabolic activities, and virulence properties of the colonizing microorganisms (Socransky 2008).

The most influencing factors on the microbiota are:

- The periodontal disease status of the host
- The pocket depth. Red complex species increased in numbers with increasing pocket depth.
- Host factors, such as smoking and diet, systemic conditions (in example diabetes and obesity) and even geographic location
- Transmission. There are two types of transmission: "vertical" (from parent to offspring) and "horizontal" (betweens individuals outside the parent offspring relationship.

Pathogenesis of periodontitis

The pathogenesis of periodontitis was described by Page and Schroeder in 1976

Kinane (2008) updated it mantaining the original 4 phases.

The progression lesion in the gingiva/periodontal tissue are: initial, early, established and advanced.

The first two describes the early stage of gengivitis, while the remaining describes the chronic gengivitis.

- the initial lesion: inflammation soon develops when plaque is formed on the gingival third of tooth surface. Classic sign of inflammation are present and the flow of gingival crevicolar fluid increases. Polymorphonucleates and lymphocytes are predominant in this phase which lasts between 2 and 4 days.
- The early lesion: after several days of plaque accumulation the vessels in the dentogingival plexus remain dilated, but their numbers increase. Thus the redness of the marginal gingiva enlarges. Again, Polymorphonucleates and lymphocytes are the predominant leukocytes in the infiltrate at this stage and very few plasma cells are noted. The basal cells of the junctional and sulcular epithelium now proliferate. A niche forms between epithelium and the enamel surface and a subgingival biofilm may now form.
- The established lesion: as the inflammation persists the lesion gets worse, due to an increased number if leucocytes. The plasma cells are predominant in old patients, while in young patients lymphocytes represent the largest part. The junctional epithelium is substituted by a pocket epithelium.
- The advanced lesion: the biofilm flourishes in the anaerobic ecological niche represented by gingival pocket. The advanced lesion is similar to established lesion, but is different in terms of loss of connective tissue attachment and alveolar bone, which are typically in this phase. The inflammatory cells arrives in the connective tissue of true attachment apparatus . Plasma cells are definitely predominant (Berglund and Donati 2005).

Bibliography

- Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005 Nov;43(11):5721-32.
- Barr C, Lopez MR, Rua-Dobles A. Periodontal changes by HIV serostatus in a cohort of homosexual and bisexual men. J Clin Periodontol. 1992 Nov;19(10):794-801.
- Bergenholtz G, Hasselgren G. Endodontics and periodontics. In: Lindhe J., ed. Clinical Periodontology and Implant Dentistry, 4th edn. Blackwell Munksgaard, Copenhagen: 2003: 318–351
- Berglund T, Donati M. Aspects of adaptive host response in periodontitis.J Clin Periodontol. 2005;32 Suppl 6:87-107.
- Block RM, Bushell A, Rodrigues H, Langeland K. A histopathologic, histobacteriologic , and radiographic study of periapical endodontic surgical specimes. Oral Surg Oral Med Oral Pathol. 1976 Nov;42(5):656-78.
- Byström A, Happonen RP, Sjögren U, Sundqvist G. Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. Endod Dent Traumatol. 1987 Apr;3(2):58-63.
- Cohen S, Blanco L, Berman L. Vertical root fractures: clinical and radiographic diagnosis. J Am Dent Assoc. 2003 Apr;134(4):434-41.
- De Moor RJ, Hommez GM, De Boever JG, Delmé KI, Martens GE. Periapical health related to the quality of root canal treatment in a Belgian population. Int Endod J. 2000 Mar;33(2):113-20.
- Dugas NN, Lawrence HP, Teplitsky PE, Pharoah MJ, Friedman S. Periapical health and treatment quality assessment of root-filled teeth in two Canadian populations. Int Endod J. 2003 Mar;36(3): 181-92.
- Ellis JS, Seymour RA, Steele JG, Robertson P, Butler TJ, Thomason JM. Prevalence of gingival overgrowth induced by calcium channel blockers: a community-based study. J Periodontol. 1999 Jan; 70(1):63-7.
- Endodontics: Colleagues for excellence. Cracking the Cracked Tooth Code. American Association of Endodontists, 1997:Chicago -
- Engström B (1964) The significance of enterococci in root canal treatment. Odontohgisk Revy 15, 87–106.
- Figdor D Microbial aetiology of endodontic treatment failure and pathogenic properties of selected species. Umea University Odontological Dissertations No. 79. Umea University, Umea, Sweden, 2002.
- Figdor D, Gulabivala K. Survival against the odds: microbiology of root canals associated with post-treatment disease. Endodontic Topics. 2006 18, 62–77.
- Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. Int J Food Microbiol. 2006 Jan 15;106(1):1-24.
- Gao Z, Flaitz CM, Mackenzie IC. Expression of keratinocyte growth factor in periapical lesions. J Dent Res. 1996 Sep;75(9):1658-63.

- Gatti JJ, Dobeck JM, Smith C, White RR, Socransky SS, Skobe Z. Bacteria of asymptomatic periradicular endodontic lesions identified by DNA-DNA hybridization. Endod Dent Traumatol. 2000 Oct;16(5):197-204.
- Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod. 2008 May;34(5):537-40. Epub 2008 Mar 4.
- Grossman LI. Origin of microorganisms in traumatized, pulpless, sound teeth. J Dent Res. 1967 May-Jun;46(3):551-3.
- Haapasalo M, Ranta K, Ranta H. Mixed anaerobic periapical infection with sinus tract. Endod Dent Traumatol. 1987 Apr;3(2):83-5.
- Haapasalo M, Shen Y, Ricucci D. Reasons for persistent and emerging post-treatment endodontic disease. Endodontic Topics. 2011; 18, 31-50
- Haapasalo M, Udnæs T, Endal U. Persistent, recurrentand acquired infection of the root canal system post-treatment. Endodontic Topics. 2003; 6, 29–56
- Hancock HH 3rd, Sigurdsson A, Trope M, Moisei-witsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001 May;91(5):579-86.
- Happonen RP. Periapical actinomycosis: a follow up study of 16 surgically treated cases. Endod Dent Traumatol. 1986 Oct;2(5):205-9.
- Harvey TE, White JT, Leeb IJ. Lateral condensation stress in root canals. J Endod. 1981 Apr;7(4): 151-5.
- Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965 Sep;20:340-9.
- Kinane DF, Chestnutt IG. Smoking and periodontal disease. Crit Rev Oral Biol Med. 2000;11(3): 356-65.
- Kinane D, Berglung T, Lindhe J. Pathogenesis of periodontitis, in Clinical Periodontology and Implant Dentistry, Lindhe J, Lang N, Karring T, cap 11, pp 285-306, 5th edition, 2008 Ed Blackweel Munksgaard, Oxford, Uk.
- Kirkevang L-L, Hörsted-Bindslev P, Ørstavik D, Wenzel A. A comparison of the quality of root canal treatment in two Danish subpopulations examined 1974– 75 and 1997–98. Int Endod J. 2001 Dec;34 (8):607-12.
- Lin LM, Langeland K. Vertical root fracture. J Endod. 1982 Dec;8(12):558-62.
- Lin LM, Wang S-L, Wu-Wang C, Chang K-M, Leung C. Detection of epidermal growth factor in inflammatory periapical lesions. Int Endod J. 1996 May;29(3):179-84.
- Lustig JP, Tamse A, Fuss Z. Pattern of bone resorption in vertically fractured endodontically treated teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000 Aug;90(2):224-7.

- Malassez ML. Sur l'existence de masses épithéliales dans le ligament alvéolodentaire chez l'homme adulte et à l'état normal. Comp Rend Soc Biol. 1884 36:241-244.
- Malassez ML. Sur le rôle débris épithélaux paradentaris: In: Travaux de l'année 1885, Laboratorie d'histologie du Collége de France. Masson G, editor. Paris: Librairie de l'Académie de Médicine, 1885 pp. 21-121.
- Meister F, Lommel TJ, Gerstein H. Diagnosis and possible causes of vertical root fractures. Oral Surg Oral Med Oral Pathol. 1980 Mar;49(3):243-53.
- Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J. 1998 Jan;31(1):1-7.
- Möller Å JR (1966) Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr. 1966 Dec 20;74(5):Suppl:1-380.
- Möller ÅJR (1966). Microbiological examination of root canals and periapical tissues of human teeth (dissertation). Göteborg, Sweden: University of Göteborg
- Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures.. Crit Rev Oral Biol Med. 2004 Nov 1;15(6):348-81.
- Nair PNR, Pajarola G, Schroeder HE. Types and incidence of human periapical lesions obtained with extracted teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996 Jan;81(1):93-102.
- Nair PN. On the causes of persistent apical periodontitis: A review. Int Endod J. 2006 Apr;39(4): 249-81.
- Nair PN, Pajarola G, Luder HU. Ciliated epithelium lined radicular cysts.Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Oct;94(4):485-93.
- Nair PN, Sjögren U, Sundqvist G. Cholesterol crystals as an etiological factor in non-resolving chronic inflammation: an experimental study in guinea pigs. Eur J Oral Sci. 1998 Apr;106(2 Pt 1): 644-50.
- Nair PN. Pathology of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds. Essential Endodontology. Oxford: Blackwell, 1998c
- Nair PNR, Schroeder HE. Epithelial attachment at diseased human tooth-apex. J Periodontal Res. 1985 May;20(3):293-300.
- Obermayr G, Walton RE, Leary JM, Krell KV. Vertical root fracture and relative deformation during obturation and post cementation. J Prosthet Dent. 1991 Aug;66(2):181-7.
- Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest. 1976 Mar;34(3):235-49.
- Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis.Int Endod J. 2001 Sep;34(6):429-34.
- Peciuliene V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of Enterococcus faecalis in previously root- filled canals in a Lithuanian population. J Endod. 2000 Oct;26(10):593-5.

- Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. (2003) Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J. 2003 Jan;36(1):1-11.
- Pitts DL, Matheny HE, Nicholls JI. An in vitro study of spreader loads required to cause vertical root fracture during lateral condensation. J Endod. 1983 Dec;9(12):544-50.
- Pitts DL, Natkin E. Diagnosis and treatment of vertical root fractures. J Endod. 1983 Aug;9(8): 338-46.
- Ricucci D, Siqueira JFJr. Apical actinomycosis as a continuum of intraradicular and extraradicular infection: Case report and critical review on its involvement with treatment failure. J Endod. 2008 Sep;34(9):1124-9.
- Rivera, Walton. Longitudinal tooth fractures: findings that contribute to complex endodontic diagnoses Endodontic Topics 2009, 16, 82–111.
- Rôcas IN, Siqueira JFJr, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod. 2004 May;30(5):315-20.
- Ross R, Nicholls J, Harrington G. A comparison of strains generated during placement of five endodontic posts. J Endod. 1991 Sep;17(9):450-6.
- Rud J, Omnell KA. Root fracture due to corrosion. Scand J Dent Res 1970: 78: 397–403
- Rundquist BD, Versluis A. How does canal taper affect root stresses? Int Endod J. 2006 Mar;39(3): 226-37.
- Sakamoto M, Siqueira JFJr, Rocas IN, Benno Y. Molecular analysis of the root canal microbiota associated with endodontic treatment failures. Oral Microbiol Immunol. 2008 Aug;23(4):275-81.
- Scapoli C, Tatakis DN, Mamolini E, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis: interleukin-1 gene cluster polymorphisms. J Periodontol. 2005 Jan;76(1):49-56.
- Sedgley C, Nagel A, Dahlén G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. J Endod. 2006 Mar;32(3):173-7.
- Sedgley C, Nagel A, Dahlén G, Reit C, Molander A. Effect of genetic variability on the inflammatory response to periodontal infection. J Clin Periodontol (2005) vol. 32 Suppl 6 pp. 72-86.
- Sidaravicius B, Aleksejuniene J, Eriksen HM. Endodontic treatment and prevalence of apical periodontitis in an adult population of Vilnius, Lithuania. Endod Dent Traumatol. 1999 Oct;15(5): 210-5.
- Simon JH. Incidence of periapical cysts in relation to the root canal. J Endod. 1980 Nov;6(11):845-8.
- Siqueira JF Jr. Endodontic infections: concepts, paradigms and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Sep;94(3):281-93.
- Siqueira JF Jr, Rôças IN. Polymerase chain reaction- based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Jan;97 (1):85-94.
- Siqueira JFJr, Rocas IN. Diversity of endodontic microbiota revisited. J Dent Res. 2009 Nov;88(11): 969-81.

- Siqueria JFJr. Periapical Actinomycosis and infection with Propionibacterium Propionicum. Endodontic Topics. 2003; 6, 78-95
- Sjögren U, Happonen RP, Kahnberg KE, Sundqvist G. Survival of Arachnia propionica in periapical tissue. Int Endod J. 1988 Jul;21(4):277-82.
- Socransky S, Haffajee D. Periodontal infection, in Clinical Periodontology and Implant Dentistry, Lindhe J, Lang N, Karring T, cap 9, pp 207-249, 5th edition, 2008 Ed Blackweel Munksgaard, Oxford, Uk.
- Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998 Feb;25(2):134-44.
- Soikkonen KT. Endodontically treated teeth and periapical findings in the elderly. Int Endod J. 1995 Jul;28(4):200-3.
- Stashenko P, Wang CY, Riley E, Wu Y, Ostroff G, Niederman R. Reduction of infection-stimulated periapical bone resorption by the biological response modifier PGG glucan. J Dent Res. 1995 Jan;74 (1):323-30.
- Stashenko P, Yu SM, Wang C-Y. Kinetics of immune cell and bone resorptive responses to endodontic infections. J Endod. 1992 Sep;18(9):422-6.
- Su L, Gao Y, Yu C, Wang H, Yu Q. Surgical endodontic treatment of refractory periapical periodontitis with extraradicular biofilm. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Jul;110(1):e40-4.
- Sunde PT, Olsen I, Debelian GJ, Tronstad L. Microbiota of periapical lesions refractory to endodontic therapy. J Endod. 2002 Apr;28(4):304-10.
- Sunde PT, Olsen I, Gobel UB, Theegarten D, Winter S, Debelian GJ, Tronstad L, Moter A. Fluorescence in situ hybridization (FISH) for direct visualization of bacteria in periapical lesions of asymptomatic root-filled teeth. Microbiology. 2003 May;149(Pt 5):1095-102.
- Sunde PT, Tronstad L, Eribe ER, Lind PO, Olsen I (2000) Assessment of periradicular microbiota by DNA-DNA hybridization. Endod Dent Traumatol. 2000 Oct;16(5):191-6..
- Sundqvist G, Figdor D, Persson S, Sjogren U (1998) Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics 85, 86-93.
- Tamse A, Fuss Z, Lustig J, Kaplavi J. An evaluation of endodontically treated vertically fractured teeth. J Endod. 1999 Jul;25(7):506-8.
- Tamse A. Iatrogenic vertical root fractures in endodontically treated teeth. Endod Dent Traumatol. 1988 Oct;4(5):190-6.
- Tamse A, Kaffe I, Lustig J, Ganor Y, Fuss Z. Radiographic features of vertically fractured endodontically treated mesial roots of mandibular molars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Jun;101(6):797-802.

- Thesleff I. Epithelial cell rests of Malassez bind epidermal growth factor intensely. J Periodontal Res. 1987 Sep;22(5):419-21.
- Trombelli L, Scapoli C, Tatakis DN, Grassi L. Modulation of clinical expression of plaque-induced gingivitis: effects of personality traits, social support and stress. J Clin Periodontol. 2005 Nov;32(11): 1143-50.
- Trombelli L, Tatakis DN, Scapoli C, Bottega S, Orlandini E, Tosi M. Modulation of clinical expression of plaque-induced gingivitis. II. Identification of "high-responder" and "low-responder" subjects. J Clin Periodontol (2004) vol. 31 (4) pp. 239-52
- Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol. 1990 Apr;6(2):73-7.
- Trope M, Rosenberg E, Tronstad L. Darkfield micro-scopic spirochete count in the differentiation of endodontic and periodontal abscesses. J Endod. 1992 Feb;18(2):82-6.
- van Winkelhoff AJ, van Steenbergen TJ, de Graaff J. The role of black-pigmented Bacteroides in human oral infections. J Clin Periodontol. 1988 Mar;15(3):145-55.
- Vidana R, Sullivan Å, Billström H, Ahlquist M, Lund B. Enterococcus faecalis infection in root canals - host-derived or exogenous source? Lett Appl Microbiol. 2011 Feb;52(2):109-15.
- von Arx T, Penarrocha M, Jensen S. Prognostic factors in apical surgery with root-end filling: A meta-analysis. J Endod. 2010 Jun;36(6):957-73.
- Walton RE, Michelich RJ, Smith GN. The histopathogenesis of vertical root fractures. J Endod. 1984 Feb;10(2):48-56.
- Walton RE, Torabinejad M. Principles and Practice of Endodontics, 3rd edn. Philadelphia: WB Saunders Co, 2002: 516
- Wilton JM, Griffiths GS, Curtis MA, Maiden MF, Gillett IR, Wilson DT, Sterne JA, Johnson NW. Detection of high-risk groups and individuals for periodontal diseases. Systemic predisposition and markers of general health. J Clin Periodontol. 1988 Jul;15(6):339-46.
- Zoletti GO, Siqueira JFJr, Santos KR (2006) Identification of Enterococcus faecalis in root-filled teeth with or without periradicular lesions by culture-dependent and independent approaches. J Endod. 2006 Aug;32(8):722-6.

5.2 immediate implant placement in infected sites: the rationale

• *Immediate implant placement*

At recent consensus workshop (Hämmerle 2004) three protocol were proposed to define the timing of implant placement:

1. *Immediate implant placement* when the implant is placed immediately after the dental extraction
2. *early implant placement* when implants are placed when soft tissue are healed (from 4 to 8 weeks);
3. *delayed implant placement* when implants are placed when the extraction site has substantially healed (from 3 to 6 months).

In the present study all the implants were inserted immediately after the extraction of the compromised tooth.

It has become common to insert implants immediately after the removal of teeth that were scheduled for extraction for various reasons. Various studies demonstrate that implants placement in fresh extraction socket in conjunction with appropriate guided bone regeneration is a predictable and reliable therapy (Becker 1990; Becker 1994a; Becker 1994b; Lazzara 1989)

This surgical approach has advantages, such as: (Barzilay 1993; Werbitt 1992; Schwartz-Arad & Chaushu 1997; Hämmerle 2004; Mayfield 1999)

- Easier definition of the implant position,
- Reduced number of visits in the dental office
- Reduced overall treatment time and costs
- Preservation of bone at the site of implantation
- Optimal soft tissue aesthetics
- Enhanced patient acceptance

Juodzbalys et al (2008) described a classification of extraction sockets based upon soft and hard tissue components, to improve the approach of the clinician to the post-extraction therapy, as summarised in the table below.

SOFT TISSUE	Extraction socket types		
Assessment	Adequate	Compromised	Deficient
Soft tissue contour variation	No	< 2mm	≥ 2 mm
Soft tissue vertical deficiency	no	1 to 2 mm	> 2mm
KG widt (mm)	> 2	1 to 2 mm	< 1
Mesial and distal papilla appearence	I	II	III
Sof tissue color, consistency ando contour	Pink, fim and smooth	Slightly red and a soft, spongy, and uneven contour	Red/bluish or red with a soft edematous and boggy or craterlike appearance
Biotype of gingival tissue (mm)	Thick (≥ 2)	Moderate (between 1 and 2 mm)	Thin (<1)

HARD TISSUE	Extraction socket types		
Assessment	Adequate	Compromised	Deficient
Height of alveolar process	> 10	Between 8 and 10	≤ 8
Available bone beyond the apex of extraction socket (mm)	≥ 4	Between 3 and 4	< 3
Extraction socket labial plate vertical position (mm)	≤ 3	Between 3 and 7	≥7
Extraction socket facial bone thickness (mm)	≥2	Between 1 and 2	< 1
Presence of socket bone lesion	No	> 10	yes
Mesial and distal intradental bone peak height (mm)	3 to 4	Between 1 and 3	<1
Mesio-distal distance between adjacent teeth (mm)	≥ 7	Between 5 and 7	≤ 5
Need for palatal angulation	< 5°	Between 5° and 30°	> 30°

Table 1 : Extraction Socket Soft and Hard Tissue Assessments and Extraction Socket Types (from Juodzbalys et al Classification of Extraction Sockets Based Upon Soft and Hard Tissue Components Journal of Periodontology 2008 Mar;79(3):413-24).

A recent literature review by Lang et al (2011) evaluated the survival and success rate of implants and implant supported prostheses, the prevalence of biological, technical and aesthetic complications, and the magnitude of soft and hard tissue changes following implant placement immediately into fresh extraction sockets.

Forty-six studies were included with a mean follow up of 2.08 years. The annual failure rate was 0.82% (95% CI: 0.48–1.39%), translating into the 2-year survival rate of 98.4% (97.3–99%).

Lang analysed five variables related to this kind of surgery: the use of antibiotics the reason for extraction, the site (maxilla/mandible and anterior/posterior), the loading and the success (biological and technical complications).

All these variables were not statistically significant except the use of antibiotics.

The annual implant failure rate in patients who were only given the single-dose of antibiotics pre-operatively was statistically significantly greater than who received 5–7 days post-operatively or a single dose pre-plus 5–7 days post-operative course of antibiotics.

It has been demonstrated in numerous animal and clinical studies in humans that following tooth extraction undisturbed wound healing will lead to loss of ridge volume and change in ridge shape.

In dogs the healing of the alveolar ridge has been well investigated and shows bone modelling and remodelling which results in horizontal and vertical resorption of the buccal socket wall (Cardaropoli, Araujo & Lindhe 2003 - Araujo & Lindhe 2005).

A recent literature review by Tan et al (Tan 2011) assessed the magnitude of dimensional changes of both the hard and soft tissues of the alveolar ridge up to 12 months following tooth extractions in humans. The search provided 3954 titles and 238 abstracts. Full text analysis was performed for 104 articles resulting in 20 studies that met the inclusion criteria. Human re-entry studies showed horizontal bone loss of 29–63% and vertical bone loss of 11–22% after 6 months following tooth extraction. These studies demonstrated rapid reductions in the first 3–6 months that was followed by gradual reductions in dimensions thereafter.

It was suggested that bone atrophy subsequent tooth extraction could be prevented by immediate implant placement.

Various study investigated this topic but the results presented shows great variability. The mean buccal bone resorption ranges between 3.14 (Botticelli, D., 2006) to 0.1 mm (Araujo & Lindhe 2011).

This high variability may be explained by the use of different pre-clinical models, different healing times, different implant diameters and geometries, as well as different surgical protocols; another confounding factor is the influence of raising a flap and exposing the underlying crestal bone (Vignoletti 2012.)

The influence of raising flap is still controversy in literature, there are a lot of study but the results mismatch and fail to point out shared indications.

Osteology group recently published a systematic review on these topics (Hämmerle 2012).

They summarised the systematic review by Lau et al (2012) and from Sanz et al (2012) who met their inclusion and exclusion criteria in the following points.

Limited to *aesthetic sites* the systematic reviews lead to following conclusions:

- Immediate implant placement leads to high implant survival rates.

- Immediate implant placement is associated with a high risk for mucosal recession. A wide range regarding the amount of recessions is reported in the literature.

Several risk factors for mucosal recession are pointed out:

- Smoking
- <1 mm buccal bone plate
- Thin soft tissue biotype
- Facial implant position
- Augmentation both of soft and hard tissue is often necessary
- In the aesthetic area, immediate implant placement should be used very restrictively

In the *posterior sites* the study from Lau et al lead to the following conclusions:

- In single tooth cases high survival and low complication rate are retrieved
- In molar sites there are situation with limited indications due to anatomical reasons
- In molar sites, soft and hard tissue augmentation are often required
- Premolar region is the most indicated site for immediate implant placement

For immediate implant placement is important the evaluation of the potential implant site. It must be evaluated carefully the morphology of the socket, in particular the slope of the axial walls, the root curvature of the extracted tooth, and the final position of the apex of the extracted tooth. A potential disadvantage with immediate implants could be the mismatch between the implant surface and the socket wall. Gaps could be present after implantation because the dental roots do not have a regular circular diameter. It is also possible that 1 or more bony socket walls will be partly resorbed either from the disease process or damaged because of a traumatic tooth extraction procedure. These potential problems have been tackled using different methods. Several manufacturers have developed new implant designs. Troncoconical-shaped or tapered implants have shown promising results even if, currently, no evidence is available proving that the tapered implant design is superior to standard cylindrical implants. (Gomez-Roman 1997 - Davarpanah 2005 - Lang 2007)

Wide-diameter implants have also been used with success to minimize the size of the gaps around implants in sockets of varying dimensions.

- *Immediate implant placement in infected sites*

As described before, immediate implant placement is a consolidated treatment when there are the right conditions.

Some studies suggest there is a contraindication with this technique when the implant is planned to be inserted in infected site (Schwartz-Arad 1997 - Becker 1990), due the risk to compromise the osseointegration (Quirynen 2003).

Nevertheless, very often teeth requiring extraction and implant placement shows periapical and/or periodontal pathology.

Alsaadi et al in a consecutively case study, found evidence in a greater implant failure rate in sites with apical lesion (Alsaadi 2007) and periodontal infection has also been related with an increased risk of implant failure. (Evian 2004 - Horwitz 2008).

Despite this, recent researches in implantology lead to place implant also in infected sites with similar outcome as in traditional technique (Del Fabbro 2009).

A recent systematic review investigates this topic (Waasdorp 2010).

They made a systematic research including data from animal and human studies, excluding animal studies that did not include a pristine control group and human case reports and case series with <1 year of follow-up. All prospective human studies were included. Studies were limited to those published in the English language, and review article data were excluded.

At the end, four animal studies and 8 human studies were included.

In the *animal study*, high implant survival rate was observed, although bone to implant contact seems to be impaired.

The data are not clear on this point (Table 2).

Study	Animal model	Number of subject	Number of implants	Type of infection	Treatment	Outcomes
Novaes et al., 1998	dog	4	28	Induced periradicular lesion versus healty socket	Debridment, rinse with tetracycline solution, antibiotic coverage	Zero failures and NSD in BIC in the experimental group.
Novaes et al., 200317	Dog	5	40 (20 non-infected controls)	ligature induced periodontitis	curettage of alveolus and antibiotics coverage	Zero failures and NSD in BIC in the experimental group (66.0% versus 62.4%).
Marcaccini et al., 200318 (same study as reference 17)	Dog	5	40 (20 non-infected controls)	ligature induced periodontitis	Fluorescein angiography of Novaes et al. 2003 cohort.17	Slower healing initially and NSD after 12 weeks.
Chang et al., 200919	Dog	4	24	induced periradicular lesion versus healty socket	Osteotomy and curettage, placement with or without membranes and antibiotic coverage	zero failures and NSD in BIC in the experimental group, and less BIC in the non membrane group

Table 2: animal studies

(From Waasdorp, J. A., Evian, C. I., & Mandracchia, M. (2010). Immediate Placement of Implants Into Infected Sites: A Systematic Review of the Literature. Journal of periodontology, 81(6), 801–808

Human studies showed high levels of implant survival rate similar to non-infected sites, even if there were a small number of studies and patients.

The treatment of the infection counted in most of the studies the use of the antibiotics (pre and/or after surgery) and an accurate debridement of the socket, as you can see in table 3.

Study/Type	Number of patients	Number of implants	Follow up (months)	Type of infection	Treatment	Outcomes	Miscellaneous
Novaes et al., 1995; case report	3	3	7 to 14	recurrent endodontic and periapical lucency	Debridment, saline rinse, GBR, and 31 days of antibiotics	100% survival	-
Villa and Rangert 2005, private-practic case series	20	97	15 to 44	endodontic and periodontic	Socket debridement, bone curettage, antibiotic irrigation, and GBR with placement. After suture, cortisone injection into soft tissue and post-surgery antibiotics. Full-arch immediate loading.	100% survival	-
Lindeboom et al., 2006;prospective, randomized trial	50	25 immediately placed into infected sites; 25 delayed placement after 3 months	12	chronic periapical pathology	Antibiotics 1 hour before surgery, socket degranulation, and GBR.	92% survival in the test group and 100% survival in the control group. NSD in soft and hard tissue parameters, except for less mid-buccal recession in the delayed group	Microbes cultured from sockets. F. nucleatum and P. micra were most prevalent.
Siegenthaler et al., 2007;controlled clinical trial	34 entered; 29 completed	29 (13 test and16 control sites with no presence of infection)	12	Periapical pathology with pain, radiolucency, fistula, suppuration, or a combination	Antibiotics 1 hour before surgery, chlorhexidine rinse, socket debridement, GBR, and antibiotics 5 days post-surger y.	100% survival and NSD in hard and soft tissue parameters between test and control sites.	Two post-surgical infections in test sites, one in control. Surgical intervention required in 1 test site and 1 control site with resolution, but decreased marginal bone levels in both.
Villa and Rangert, 2007;private-practice case series	33	100 maxillary (24 in healed sites)	12	endodontic and periodontic or root fracture	Socket debridement, bone curettage, antibiotic irrigation, and GBR with placement. After suture, cortisone injection into soft tissue, post-surgery antibiotics. Full-arch immediate loading	97.4% survival	-
Casap et al., 2007;case series	20	30	12 to 72	Subacute periodontal, chronic periapical, chronic perioendodontic, chronic periodontal, and a periapical cyst	14 days of antibiotics, debridement, peripheral intrasocket ostectomy, GBR, and primary closure.	97.7% survival	One pseudomembranous colitis, one membrane exposure, and one deficiency of attached gingiva.

Study/Type	Number of patients	Number of implants	Follow up (months)	Type of infection	Treatment	Outcomes	Miscellaneous
Naves et al., 2009; case report	1	3	36	chronic periapical pathology	Antibiotics 1 hour before surgery and 7 days post-surgery, apical access flap with debridement of lesion, and GBR.	100% survival	-
Del Fabbro et al 2009	30	61	10 to 21	chronic periapical (histologic granuloma)	socket debridment and PRGF coating of implant	98.45% survival	-

Table 3: human studies

(From Waasdorp, J. A., Evian, C. I., & Mandracchia, M. (2010). Immediate Placement of Implants Into Infected Sites: A Systematic Review of the Literature. Journal of periodontology, 81(6), 801–808

The limit of this studies was to lack of information of the infection affecting the extracted teeth. Furthermore, there is no relation between type of infection and outcome in terms of implant survival rate. It could be interesting in future studies to obtain histopathologic data, and evaluate how much periapical and/or periodontal pathology can affect outcome of immediate implant therapy in infected sites.

The physiologic healing process after tooth extraction normally solves residual infection, but as an infection increases inflammatory activity, infection may result in increased bone resorption and a higher risk of implant stability loss and failure. The presence of granulation tissue in the socket of an infected tooth must be considered as an inflammatory response to bacteria. This reactive tissue protects bone from direct bacterial aggression and, if carefully removed, will reveal healthy bone. Therefore, infected tooth extraction and conventional granulation tissue removal, as well as an early onset of antibiotic treatment, may be effective in reducing the inflammatory response and the consequent bone resorption activity (Villa & Rangert 2007).

Alternative to immediate implant placement

When a tooth is extracted, the surrounding bone loses his primary function and undergoes to process of modelling and remodelling which results in a reduction on the overall ridge dimensions with significant changes in both the buccal and lingual bone crests.

There are a lot of studies investigating this process and nowadays there is evidence that the most resorption take part during the first three months of healing but continues all over the first year. Schropp in 2003 demonstrated that there is 50% resorption of the bucco-lingual dimension of the alveolar ridge; Araujo and Lindhe (2005) identified in the buccal bone plate the main alveolar side to be resorbed.

The resorption of the alveolar bone would be a problem in a subsequent rehabilitation with dental implants.

To avoid this problem related to early changes in alveolar bone after extraction, some techniques called "socket preservation" are proposed. These techniques permit to preserve as much as possible the residual alveolar bone.

Vignoletti et al (2011) systematically review the effects of these technique to evaluate their outcome.

The techniques evaluated in this study were:

- filling the socket with autologous bone grafts or bone substitutes (allogenic, xenogenic and synthetic grafts);
- isolating the socket with the use of barrier membranes, soft tissue autografts or soft tissue substitutes (allogenic and others),
- promoting the healing process of the socket by the addition of growth factors or bone morphogenetic proteins.

These techniques (test group) are compared to spontaneous healing of the socket (healing group).

The search strategy retrieved 296 articles. After applying inclusion ad exclusion criteria, finally 14 studies were included.

In terms of vertical bone loss the control group shows a range comprised between -0.3 and -3.75 mm, while test group shows from -2.48 to 1.3 mm.

This meta-analysis found that different ridge preservation procedures permits a significantly less vertical and horizontal contraction of the alveolar bone crest.

Due to the heterogeneity of the studies included, this review does not indicate which technique is the most reliable, but better results are obtained through the use of barrier membranes, a flap surgical procedure and a full flap closure.

BIBLIOGRAPHY

- Alsaadi G, Quirynen M, Komarek A, van Steenberghe D. Impact of local and systemic factors on the in- cidence of oral implant failures, up to abutment connection. J Clin Periodontol. 2007 Jul;34(7): 610-7. Epub 2007 Apr 13.
- Araujo, M. G. & Lindhe, J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol. 2005 Feb;32(2):212-8.
- Araujo, M. G. & Lindhe, J. Socket grafting with the use of autologous bone: an experimental study in the dog. Clin Oral Implants Res. 2011 Jan;22(1):9-13.
- Barzilay, I. Immediate implants: their current status. Int J Prosthodont. 1993 Mar-Apr;6(2):169-75.
- Becker W, Becker BE. Guided tissue regeneration for implants placed into extraction sockets and for implant dehiscences: Surgical techniques and case report. Int J Periodontics Restorative Dent. 1990;10(5):376-91.
- Becker W, Dahlin C, Becker BE, Lekholm U, van Steenberghe D, Higuchi K, Kultje C. The use of e-PTFE barrier membranes for bone promotion around titanium implants placed into extraction sockets: a prospective multicenter study. Int J Oral Maxillofac Implants. 1994 Jan-Feb;9(1):31-40.
- Becker WBB: Bone promotion arounde-PTFE augmented implants placed in immediate extraction sockets. In Buser D, Dahlin C, Schenk RK (eds): Guided Bone Regeneration in Implant Dentistry. Chicago, IL, Quintessence, 1994, pp. 137-154
- Becker W, Becker BE, Handlesman M, Celletti R, Ochsenbein C, Hardwick R, Langer B. Bone formation at dehisced dental implant sites treated with implant augmentation material: a pilot study in dogs. Int J Periodontics Restorative Dent. 1990;10(2):92-101.
- Botticelli D, Persson LG, Lindhe J, Berglundh T. Bone tissue formation adjacent to implants placed in fresh extraction sockets: an experimental study in dogs. Clin Oral Implants Res. 2006 Aug;17(4): 351-8.
- Cardaropoli G, Araujo M. Lindhe, J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J Clin Periodontol. 2003 Sep;30(9):809-18.
- Casap N, Zeltser C, Wexler A, Tarazi E, Zeltser R. Immediate placement of dental implants into debrided infected dentoalveolar sockets. J Oral Maxillofac Surg. 2007 Mar;65(3):384-92.
- Chang SW, Shin SY, Hong JR, Yang SM, Yoo HM, Park DS, Oh TS, Kye SB. Immediate implant placement into infected and noninfected ex- traction sockets: A pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Feb;107(2):197-203.
- Davarpanah M, Caraman M, Szmukler-Moncler S, Jakubowicz-Kohen B, Alcolforado G. Preliminary data of a prospective clinical study on the Osseotite NT implant: 18-Month follow-up. Int J Oral Maxillofac Implants. 2005 May-Jun;20(3):448-54.
- Del Fabbro M, Boggian C, Taschieri S. Immediate implant placement into fresh extraction sites with chronic periapical pathologic features combined with plasma rich in growth factors: J Oral Maxillofac Surg. 2009 Nov;67(11):2476-84.

- Evian CI, Emling R, Rosenberg ES, Waasdorp JA, Halpern W, Shah S, Garcia M. Retrospective analysis of implant survival and the influence of periodontal disease and immediate placement on long-term results. Int J Oral Maxillofac Implants. 2004 May-Jun;19(3):393-8.
- Gomez-Roman G, Schulte W, d'Hoedt B, Axman-Krcmar D. The Frialit-2 implant system: Five-year clinical experience in single-tooth and immediately postextraction applications. Int J Oral Maxillofac Implants. 1997 May-Jun;12(3):299-309.
- Hämmerle CHF, Araújo MG, Simion M, Osteology Consensus Group 2011. Evidence-based knowledge on the biology and treatment of extraction sockets. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:80-2.
- Hämmerle CHF, Chen ST, Wilson TG. Consensus statements and recommended clinical procedures regarding the placement of implants in extraction sockets Int J Oral Maxillofac Implants. 2004;19 Suppl:26-8.
- Horwitz J, Zuabi O, Machtei E. Radiographic changes around immediately restored dental implants in peri- odontally susceptible patients: 1-year results. Int J Oral Maxillofac Implants. 2008 May-Jun;23(3): 531-8.
- Juodzbalys G, Sakavicius D, Wang HL. Classification of Extraction Sockets Based Upon Soft and Hard Tissue Components J Periodontol. 2008 Mar;79(3):413-24.
- Lang NP, Tonetti MS, Suvan JE, Pierre Bernard J, Botticelli D, Fourmousis I, Hallund M, Jung R, Laurell L, Salvi GE, Shafer D, Weber HP; European Research Group on Periodontology. Immediate implant placement with transmucosal healing in areas of aesthetic priority: A multicenter randomized-controlled clinical trial I. Surgical outcomes. Clin Oral Implants Res. 2007 Apr;18(2): 188-96.
- Lang NP, Pun L, Lau KY, Li KY, Wong, MC. A systematic review on survival and success rates of implants placed immediately into fresh extraction sockets after at least 1 year. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:39-66.
- Lazzara RJ. Immediate implant placement into extraction sites: surgical and restorative advantages. Int J Periodontics Restorative Dent. 1989;9(5):332-43.
- Lindeboom JA, Tjiook Y, Kroon FH. Immediate placement of implants in periapical infected sites: A pro- spective randomized study in 50 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Jun;101(6):705-10.
- Marcaccini AM, Novaes AB Jr., Souza SL, Taba M Jr., Grisi MF. Immediate placement of implants into periodontally infected sites in dogs. Part 2: A fluorescence microscopy study. Int J Oral Maxillofac Implants. 2003 Nov-Dec;18(6):812-9.
- Mayfield (1999) Immediate and delayed submerged and transmucosal implants. 3rd European Workshop on Periodontology, Ittingen Switzerland

- Naves Mde M, Horbylon BZ, Gomes Cde F, Menezes HH, Bataglion C, Magalhaes D. Immediate implants placed into infected sockets: A case report with 3-year follow-up. Braz Dent J. 2009;20(3): 254-8.
- Novaes AB Jr., Marcaccini AM, Souza SL, Taba M Jr., Grisi MF. Immediate placement of implants into periodontally infected sites in dogs: A histomorphometric study of bone-implant contact. Int J Oral Maxillofac Implants. 2003 May-Jun;18(3):391-8.
- Novaes AB Jr., Novaes AB. Immediate implants placed into infected sites: A clinical report. Int J Oral Maxillofac Implants. 1995 Sep-Oct;10(5):609-13.
- Novaes AB Jr., Vidigal Junior GM, Novaes AB, Grisi MF, Polloni S, Rosa A. Immediate implants placed into infected sites: A histomorphometric study in dogs. Int J Oral Maxillofac Implants. 1998 May-Jun;13(3):422-7.
- Quirynen M, Gijbels F, Jacobs R. An infected jawbone site compromising successful osseointegration. Periodontol 2000. 2003;33:129-44.
- Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent. 2003 Aug;23(4):313-23.
- Schwartz-Arad D, Chaushu G. The ways and wherefores of immediate placement of implants into fresh extraction sites: A literature review. J Periodontol. 1997 Oct;68(10):915-23.
- Schwartz-Arad D, Chaushu G. The ways and wherefores of immediate placement of implants into fresh extraction sites: a literature review. J Periodontol. 1997 Oct;68(10):915-23.
- Siegenthaler DW, Jung RE, Holderegger C, Roos M, Hammerle CH. Replacement of teeth exhibiting peri- apical pathology by immediate implants: A prospective, controlled clinical trial. Clin Oral Implants Res. 2007 Dec;18(6):727-37.
- Tan WL, Wong, TLT, Wong MCM, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:1-21.
- Vignoletti, F, Discepoli N, Müller A, De Sanctis M, Muñoz F, Sanz M. Bone modelling at fresh extraction sockets: immediate implant placement versus spontaneous healing: an experimental study in the beagle dog. J Clin Periodontol. 2012 Jan;39(1):91-7.
- Vignoletti F, Matesanz P, Rodrigo D, Figuero E, Martin C, Sanz M 2011. Surgical protocols for ridge preservation after tooth extraction. A systematic review. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:22-38
- Villa R, Rangert B. Early loading of interforaminal implants immediately installed after extraction of teeth presenting endodontic and periodontal lesions. Clin Implant Dent Relat Res. 2005;7 Suppl 1:S28-35.
- Villa R, Rangert B. Immediate and early function of implants placed in extraction sockets of maxillary infected teeth: A pilot study. J Prosthet Dent. 2007 Jun;97(6 Suppl):S96-S108.

- Waasdorp JA, Evian CI, Mandracchia M. Immediate Placement of Implants Into Infected Sites: A Systematic Review of the Literature. J Periodontol. 2010 Jun;81(6):801-8.
- Werbitt MJ, Goldberg PV. The immediate implant: bone preservation and bone regeneration. Int J Periodontics Restorative Dent. 1992;12(3):206-17.

5.3 PRGF AND IMPLANT DENTISTRY

Recent research in medicine investigate the field of "endogenous regenerative medicine", in which patient's own plasma and platelet-derived cytokines and biologically active factors are used to stimulate wound healing and tissue regeneration (Anitua, 1999, 2006, 2008).

PRGF is a part of this platelet-derived factors.

PRGF develops its potential through two different processes, as exposed in 2012 from Anitua et al (Anitua 2012). The first is the release of hundreds of proteins and growth factors from platelets that actively stimulate tissue regeneration. This pool of factors is added to the biologically active molecules already present in human plasma. Second is the formation of a three-dimensional fibrin matrix that retains and later releases part of the growth factors, and which also acts as a temporal nesting scaffold for the cells.

PRGF technology would use the potential of the growth factors contained in the platelets. When activated platelets release a pool of substances capable to enhance the recruitment, growth and morphogenesis of cells.

The growth factors stored in the platelets are summarised in the table below:

GROWTH FACTORS	DESCRIPTION
Platelet derived growth factors (PDGF)	Activate mesenchymal cells like fibroblasts, osteoblasts and adipocytes, stimulates the formation of collagen type I. It also promotes angiogenesis indirectly by activating macrophages
Beta- transforming growth factor (TGF-B)	Stimulates proliferation and differentiation of mesenchymal stem cells and promotes the synthesis of collagen type I by osteoblasts. It's angiogenic and inhibit the formation of osteoclasts and the proliferation of epithelial cells in the presence of other growth factors
Epidermal growth factor (EGF)	Mitogenic and chemotactic effect on fibroblasts and epithelial cells. Inductor for cell migration and stimulator for the formation of granulation tissue
Vascular endothelial growth factor (VEGF)	Induces the chemotaxis and proliferation of endothelial cells to promote angiogensis and to provoke the hiperpermeability of blood vessels. It is mitogenic, proapoptotic and promotes the chemotaxis and differentiation of epithelial, renal and glial cells as well as fibroblasts
Basic-fibroblastic growth factor (b-FGF)	Stimulates and coordinates the mitogenesis of mesenchymal stem cells during growth, maintenance and tissue repair. Such effects were reported for fibroblasts, osteoblasts, chondrocytes, smooth muscle cells and skeletal myoblasts. Angiogenesis is also enhanced through endothelial cell stimulation to undergo mitosis and migration
Insulin-like growth factor-I (IGF-I)	It stimulates the formation of bone matrix by promoting pre-osteoblast proliferation and stimulates the synthesis of osteocalcin, alkaline phosphatase and collagen type I by osteoblasts. It also stimulates the proliferation and differentiation of mesenchymal stem cells in chondrogenesis, adipogenesis, myogenesis, promotes neuronal differentiation and induces a chemotactic effect on vascular endothelial cells.
Platelet Factor 4 (PF4)	Negative regulator of angiogenesis and as a powerful inhibitor of endothelial cell proliferation. It is a chemotactant for neutrophils and fibroblasts

GROWTH FACTORS	DESCRIPTION
ATP and ADP	Regulation of a variety of functions in many tissues
Angiopoietin-2 (Ang2)	It works in concert with VEGF to promote neoangiogenesis as it destabilizes the existing vessels.
Fibronectin	It supports cell growth and migration into the clots and participates in the elaboration of the extracellular matrix that will replace the clot. Moreover, it mediates the growth and migration of cells within the matrix
Osteocalcin (OC)	Function as a localization site for hydroxyapatite crystals during bone matrix synthesis
Serotonin	Hemostasis
Thrombospondin-1 (TSP-1)	It results in the formation of multi- protein complexes at the cell surface to modulate the cellular phenotype.

Table 1: growth factors and a brief description (from Anitua, E, Alkhraisat, MH, Orive, G. (2012). Perspectives and challenges in regenerative medicine using plasma rich in growth factors. Journal of Controlled Release, 157(1), 29–38.)

Fibrine plays a fundamental role in the formation of the scaffold of PRGF through the formation of a three-dimensional fiber network.

Fibrin network presents also visco-elastic properties. Fibrin clots experimented with shearing deformations at different frequencies simulating slow and fast deformations, indicated that fine clot maintained its stiffness over the tested frequencies, whereas coarse clot stiffness changed to some degree as function of frequency (Gerth, 1974 - Roska, 1982).

Fibrin has a biological importance in interacting with the surrounding tissue, i.e. Extracellular matrix and cellular components.

It has been considered that the clinical efficacy of platelet rich plasma preparations may be related in part to the pattern of growth factors release from fibrin hydrogel. Platelets start actively releasing growth factors within the first 10 min of activation and secrete 90–95% of their presynthesized growth factors within 1 h (Marx 2004). Several biomaterials have been employed to prolong the release of growth factors from platelet rich plasma preparations. Gelatin, alginate, collagen and calcium sulfate are examples of such materials (Chen 2010). It has been indicated that the retention of cytokines by bone substitute materials was related to material's specific surface area (Klein, 2010).

In this study PRGF has been used in adjunction to implant therapy to enhance the wound healing, the osseointegration process and to minimise the post-surgery discomfort. The biologic properties of PRGF exploit the potential of several platelet growth factors (platelet-derived growth factor, transforming growth factor-β, endothelial growth factor, vascular endothelial growth factor, insulin-like growth factor-1, basic fibroblast growth factor, hepatocyte growth factor), obtained with a simple centrifugation procedure, to stimulate several biological functions such as chemotaxis, angiogenesis, proliferation, differentiation, modulation, thereby representing a possible therapeutic device for a more rapid and effective regeneration of hard and soft tissues (Giannobile 1996. - Intini 2009. - Lind 1998)

PRGF has been recently proposed in the field of oral surgery.

Different PRGF formulations are available and have different therapeutic indications (Anitua 2007).The liquid PRGF (Plasma Very Rich in Growth Factors - PVRGF) can be used to bioactivate the surface of dental implants (Anitua 2006) and to enhance soft tissue healing. The scaffold-like PRGF, with a gel consistency, has the potential to promote bone regeneration when associated with other osteoconductive materials. Furthermore, it may act as a glue for granular bone substitutes, preventing their dispersion and improving graft handling properties and adaption to filling bone defects. Elastic fibrin can be use to seal peri-implant defects in post-extraction sites and, in general, as a natural barrier membrane in all guided bone regeneration procedures (Anitua 2009, 2010).

PRGF accelerates the regeneration of bone and soft tissue. A recent study assess that the addition of platelet-rich preparation to Deprotenized Bovine Bone increases the bone density in noncritical size defects in rabbits (Aghaloo 2004).

In a similar study, Suba et al found that platelet rich factor in conjunction with β-tricalcium phosphate increased the bone area percentage 6 weeks after grafting compared with the biomaterial alone. The difference could be appreciated after 12 week, meanwhile there is no difference after 24 week. This could be explained by the idea that platelet rich in growth factor is to accelerate bone regeneration (Suba 2004).

Another interesting focus of interest lies on the combination of PRGF with dental implants with the aim of facilitating the bone–implant contact of the latter. The development of novel surfaces with increased osseointegration potential is garnering increased attention in the field of oral implantology. The humidification of titanium rough implant surfaces with activated liquid PRGF enables the formation of a biological nano-membrane (bioactivation of dental implants) composed of a fibrin scaffold containing fibronectin, osteonectin and vitronectin which provide specific sites for cell adhesion. Moreover, the release of the large list of growth factors from the activated platelets embedded within the fibrin scaffold will also promote accelerated bone apposition on the implant surface (Anitua 2007).

Recently, it has been observed that humidification of dental implants with liquid PRGF enhances the percentage of bone–implant contact in compared with implants without the biological preparation (Anitua 2006). In addition, explantation of the implants revealed that the whole surface of the PRGF-treated implants was covered by newly formed bone whereas only the upper half was surrounded in control implants (Anitua 2006).

This findings are confirmed by recent studies showing a survival rate superior to 99.2% in pool of 5700 implants inserted in 1060 patients (Anitua, 2008).

PRGF could be used also in sinus lift procedures.

For example, the sinus lateral approach involves the separation of a sinus bone window, which will be placed in its original anatomic position in a posterior phase of the surgery. Placing this bone window in the pool of biologically active growth factors may maintain the viability and functionality of the bone

tissue. In the same approach, the autologous and biocompatible fibrin scaffold may be used as autologous sealant biomaterial in the case of Schneiderian membrane perforation. Furthermore, this scaffold can be easily combined with any bone augmentation biomaterial to create the final graft. This mixture facilitates the manipulation and administration of the graft, increasing the biosafety of the approach (E. Anitua, 2009).

Taschieri et al (2012.) has recently published a pilot study which evaluate the potential effect on PRGF for the management of the Schneiderian membrane during maxillary sinus lift: despite the limitations of the study, concerning the sample size and the study design, the use of PRGF may be helpful in reducing complications following sinus lift surgery.

PRGFs could be particularly indicated for immediate postextraction implants; combined with minute bone chips obtained during drilling procedure, it could fill the gap between the implant surface and socket walls, providing an osteoconductive, autologous graft that replaces and improves the bone substitutes commonly used (Del Fabbro 2009).

Bibliography

- Aghaloo TL, Moy PK, Freymiller EG. Evaluation of platelet rich plasma in combination with anorganic bovine bone in the rabbit cranium: a pilot study. Int J Oral Maxillofac Implants. 2004 Jan-Feb;19(1):59-65.
- Anitua E, Alkhraisat MH, Orive, G. Perspectives and challenges in regenerative medicine using plasma rich in growth factors. J Control Release. 2012 Jan 10;157(1):29-38.
- Gerth C, Roberts WW, Ferry JD. Rheology of fibrin clots: II. Linear viscoelastic behavior in shear creep. Biophys Chem. 1974 Oct;2(3):208-17.
- Anitua E, Orive G, Aguirre JJ, Ardanza B, Andía I. 5-year clinical experience with BTI dental implants: risk factors for implant failure. J Clin Periodontol. 2008 Aug;35(8):724-32.
- Anitua EA. Enhancement of osseointegration by generating a dynamic implant surface.J Oral Implantol. 2006;32(2):72-6.
- Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials. 2007 Nov;28(31):4551-60.
- E. Anitua, M. Sánchez, A.T. Nurden, P. Nurden, G. Orive, I. Andía. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006 May;24(5):227-34.
- Anitua E, Sánchez M, Orive G, Andia I. Delivering growth factors for therapeutics, Trends Pharmacol Sci. 2008 Jan;29(1):37-41
- Anitua E, Prado R, Orive G. Bilateral Sinus Elevation Evaluating Plasma Rich in Growth Factors Technology: A Report of Five Cases. Clin Implant Dent Relat Res. 2012 Mar;14(1):51-60.
- E. Anitua. Plasma rich in growth factors: preliminary results of use in the preparation of sites for implants. Int J Oral Maxillofac Implants. 1999 Jul-Aug;14(4):529-35.
- E. Anitua, R. Prado, G. Orive. A new lateral approach for sinus elevation using PRGF technology, Clin Implant Dent Relat Res. 2009 Oct;11 Suppl 1:e23-31
- Roska FJ, Ferry JD. Studies of fibrin film. I. Stress relaxation and birefringence, Biopolymers 21. Biopolymers. 1982 Sep;21(9):1811-32.
- Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF. A review on endogenous regenerative technology in periodontal regenerative medicine. Biomaterials. 2010 Nov;31(31):7892-927.
- Del Fabbro M, Boggian M, Taschieri S. Immediate Implant Placement Into Fresh Extraction Sites With Chronic Periapical Pathologic Features Combined With Plasma Rich in Growth Factors: Preliminary Results of Single-Cohort Study. J Oral Maxillofac Surg. 2009 Nov;67(11):2476-84.
- Giannobile WV. Periodontal tissue engineering by growth factors. Bone. 1996 Jul;19(1 Suppl): 23S-37S.
- Intini G. The use of platelet-rich plasma in bone reconstruction therapy. Biomaterials. 2009 Oct;30 (28):4956-66.
- Lind M. Growth factor stimulation of bone healing. Effects on osteoblasts, osteotomies, and implants fixation. Acta Orthop Scand Suppl. 1998 Oct;283:2-37.

- Klein MO, Kämmerer PW, Scholz T, Moergel M, Kirchmaier CM, Al-Nawas B. Modulation of platelet activation and initial cytokine release by alloplastic bone substitute materials. Clin Oral Implants Res. 2010 Mar;21(3):336-45.
- Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004 Apr;62(4): 489-96.
- Suba Z, Takács D, Gyulai-Gaál S, Kovács K. Facilitation of b- tricalcium phosphate induced alveolar bone regeneration by platelet rich plasma in beagle dogs: a histologic and histomorphometric study. Int J Oral Maxillofac Implants. 2004 Nov-Dec;19(6):832-8.
- Taschieri S, Corbella S, Del Fabbro M. Use of Plasma Rich in Growth Factor for Schneiderian membrane management during maxillary sinus augmentation procedure. J Oral Implantol. 2012 Mar 12. (Epub ahead of print)

6. Conclusion

The aim of this study was to assess the survival rate of implants placed in post-extractive infected sites with PRGF.

The patients who met the inclusion and exclusion criteria were 20 and the implants inserted were 29.

The survival rate at 1 year follow-up (started at the end of prosthetic phase) was 100%.

The overview about post-extractive implant evidenced how this approach has become a reliable technique, with high survival rate.

Nevertheless it is very important an appropriate study of each case, evaluating all the factors which could affect the outcome. Despite the high survival rate of immediate implant placement, in anterior sites there are high risk in terms of mucosal recession. Smoking, thin soft tissue biotype, a facial implant position, are risk factors, as an augmentation of soft and hard tissue procedure which is often required. Thus, in the aesthetic area, immediate implant placement should be used very restrictively (Hammerle 2012).

In posterior sites, in single tooth cases, high survival and low complication rate are retrieved; in molar sites there are situation with limited indications due to anatomical reasons and soft and hard tissue augmentation are often required; premolar region is the most indicated site for immediate implant placement (Lau 2012).

In 1995 Novaes started placing implants immediately after extraction of teeth with recurrent endodontic and periapical lucency. In recent years several study investigated this topic, to prove that implantology could minimise time and procedures with implants inserted in post-extractive infected sites.

The implant survival rates in all this studies is high; the sites must be thoroughly debrided prior to placement and guided bone regeneration is usually performed to fill the bone–implant gap and/or socket deficiencies. Although controversial, systemic antibiotics should be used. Nevertheless there is a great heterogeneity in the protocols adopted. Especially there is a lack of histopatologic datas, to compare and classify lesions of different origin.

This thesis, showed using a critical literature analysis how many bacterial species lead to a periodontal or endodontic infection and the pathogenesis interaction between them.

There is not a clear association between histologic data and clinical signs and symptoms, and between endodontic or periodontal lesion and implant survival rate.

Future researches should investigate this unanswered questions, to establish shared protocols to treat patients with hopeless teeth due to periodontal or endodontic lesion in the most reliable way.

Patient's own plasma and platelet-derived cytokines and biologically active factor has become recently a field of interest for researchers in different surgical disciplines and in dentistry too due to their capacity to improve wound healing and tissue regeneration.

In the clinical research part of this thesis, the high implant survival rate suggests the usefulness of platelets concentrate.

This confirms the findings of others recently published studies which assess the use of PRGF from single unit implant in healed sites to sinus floor elevation (Anitua 2008, Taschieri 2012).

Furthermore, in patients treated with platelets concentrate, whereas quality of life has been evaluated, significantly good results are retrieved (Del Fabbro 2009, Taschieri 2012).

Bibliography

- Anitua E, Orive G, Aguirre JJ, Ardanza B, Andía I. 5-year clinical experience with BTI dental implants: risk factors for implant failure. J Clin Periodontol. 2008 Aug;35(8):724-32.
- Del Fabbro M, Boggian M, Taschieri S. Immediate Implant Placement Into Fresh Extraction Sites With Chronic Periapical Pathologic Features Combined With Plasma Rich in Growth Factors: Preliminary Results of Single-Cohort Study. J Oral Maxillofac Surg. 2009 Nov;67(11):2476-84
- Hämmerle CHF, Araújo MG, Simion M, Osteology Consensus Group 2011. Evidence-based knowledge on the biology and treatment of extraction sockets. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:80-2.
- Novaes AB Jr., Novaes AB. Immediate implants placed into infected sites: A clinical report. Int J Oral Maxillofac Implants. 1995 Sep-Oct;10(5):609-13.
- Tan WL, Wong, TLT, Wong MCM, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res. 2012 Feb;23 Suppl 5:1-21.
- Taschieri S, Corbella S, Del Fabbro M. Use of Plasma Rich in Growth Factor for Schneiderian membrane management during maxillary sinus augmentation procedure. J Oral Implantol. 2012 Mar 12. (Epub ahead of print)