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# THE EFFECT OF LASER TREATMENT ON ALVEOLAR BONE PRESERVATION AND IMMEDIATE IMPLANT PLACEMENT - RANDOMIZED CONTROLLED TRIAL

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# ABSTRACT

Among the different causes for implant failure, most of which being clinical aspects of the implant treatment, medications and health conditions, bacterial periodontal infections have been reported to be a major risk factor for early and late loss of dental implants. In addition to having been associated with the etiology of peri-implantitis and periodontitis, post-extraction periodontal pathogens have been linked to increase in bone loss, reduction in bone healing and implant instability.

Current methods for post-extraction disinfection of the implant site are mainly based on the use of drugs and surgical techniques, although the pharmacological risk of the former and the invasiveness of the latter have led researchers to seek alternative therapeutic methods which are safer for the patients, and one of them is laser therapy.

The present work outlines a new laser protocol consisting of degranulation, disinfection, decortication, de-epithelialization, clot stabilization and photobiomodulation using Er:YAG and Nd:YAG wavelengths with immediate implant placement, which has been compared to a standard post-extraction protocol with immediate implant placement, in a randomized clinical trial comprising 14 patients attending the combined implantology/oral surgery outpatient clinic of Clitrofa – Centro Médico, Dentário e Cirúrgico Lda, in Trofa - Portugal, between July 2022 and February 2023.

For the evaluation and comparison of patients in the control and experimental groups, the following measurement techniques have been used: (I) measurements of alveolar bone loss and density using cone-beam computed tomography (CBCT), (II) implants insertion torque (IT), (III) implants resonance frequency analysis (RFA) and (IV) clinical side effects. Results have shown the existence of statistically significant differences between the laser and the standard post-extraction procedures and therefore reinforce the potential of application of laser treatment in implant placement.

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To my family for keeping my spirits and hopes always above ground and for being there when I needed it.

This thesis is dedicated to my beloved wife Carina Ramos and to my two children Henrique Mar and Bruna Mar, all together they are my life.

## DECLARATION

*"Except for the help listed in the Acknowledgements, the contents of this thesis are entirely my own work. This work has not been previously submitted, in part or in full, for a degree or diploma of this or any other University or examination board".*

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next-generation sequencing (NGS)
gingival crevicular fluid (GCF)
bleeding on probing (BOP)
probing pocket depth (PPD)
periodontal ligament (PDL)
polymerase chain reaction (PCR)
fluorescence in situ hybridization (FISH)
checkerboard DNA-DNA hybridization (CKB)
next-generation sequencing (NGS)
bone morphogenetic protein (BMP)
guided bone regeneration (GBR)
resonance frequency analysis (RFA)
near-infrared (NIR)
photobiomodulation (PBM)
neodymium-doped yttrium aluminum garnet (Nd:YAG)
erbium-doped yttrium aluminium garnet laser (Er:YAG)
dental implant failure (DIF)
cone-beam computed tomography (CBCT)
insertion torque (IT)
hounsfield units (HU)
multislice computed tomography (MSCT)
field of view (FOV)
implant stability quotient (ISQ)
bone-implant contact (BIC)

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# CHAPTER 1 – INTRODUCTION

Since the late 70s, the use of dental implants to replace missing teeth has become an increasingly common clinical practice and continuous technological innovations have now made implant therapy more reliable and accessible to the population (Gensi *et al*., 2020). Approximately 12 million implants are placed worldwide every year, but the past three decades have seen the emergence of two new oral diseases: peri-implantitis, which affects both the soft and hard tissues surrounding the implant; and mucositis, which precedes peri-implantitis and, instead, involves only the soft tissues (Albrektsson *et al.*, 2014). Mucositis affects >50% of the implants, while almost 20% of implants develop peri-implantitis (Berglundh *et al*., 2018). About half of all inserted dental implants are thus suffering from diseases leading, in most cases, to implant loosening or the need for implant removal with very large clinical and socio-economical burdens and serious impairment of patients' quality of life (Berglundh *et al*., 2018; Gensi *et al*., 2020).

Risk factors related to the development of peri-implant disease reported in the literature are: smoking, genetic factors such as a combined IL-1 genotype positivity, history of periodontitis, poor oral hygiene, systemic diseases (uncontrolled diabetes mellitus, cardiovascular and immunodepressive diseases), iatrogenic causes (such as extra cement), poor peri-implant soft tissue quality (keratinized gingiva thickness < 2 mm), history of one or more implant losses, excessive occlusal loading, and titanium particles (Butera *et al.,* 2022).

The disease is the result of continuous inflammation, tissue destruction, and microbial pressure. Similarly, to those known as periodontal diseases, these factors are also influenced by host-specific immunemediated response and genetics and are partially modulated by lifestyle and environmental factors (Gensi *et al*., 2020). Despite significant advances made in both periodontal microbiology and pathobiology, it is yet unclear whether the primary disease trigger is the microbial challenge or the hyperinflammatory state itself (Van Dyke *et al.,* 2020). However, unlike the case of bacteria associated with periodontal diseases that have been studied for decades, the only recent emergence of peri-implant diseases limited similar investigations for mucositis and peri-implantitis. Although some known periodontitis-associated bacteria may also be connected with peri-implant diseases, different microorganisms have been suggested to be involved in these two clinically distinct conditions (Van Dyke *et al.,*

2020). A thorough profiling of the microbiome associated with peri-implant diseases is thus an important step to undertake, to then help better contextualize host response, genetics and environmental factors, and start moving toward the development of diagnostic, preventive, and therapeutic approaches (Gensi *et al*., 2020).

Therefore, effective methods of reducing bone loss, accelerating bone healing, and increasing predictability, are actively sought. Most studies focus on drugs or surgical techniques but other modalities affecting the healing process have been investigated; among them is the use of laser therapy (Noba *et al*., 2018).

This research project was designed to evaluate a comprehensive post-extraction with immediate implant placement laser protocol versus post-extraction with immediate implant placement standard protocol.

This laser protocol consists of degranulation, disinfection, decortication, de-epithelialization, clot stabilization and photobiomodulation using Er:YAG and Nd:YAG wavelengths.

The 14 patients included in this randomized clinical trial attended the combined implantology/oral surgery outpatient clinic of Clitrofa – Centro Médico, Dentário e Cirúrgico Lda, in Trofa - Portugal, between July 2022 and February 2023.

This investigation’s results were subdivided into 4 outcomes: (I) measurements of alveolar bone loss and density using cone-beam computed tomography (CBCT), (II) implants insertion torque (IT), (III) implants resonance frequency analysis (RFA) and (IV) clinical side effects.

# CHAPTER 2 – GENERAL LITERATURE REVIEW

## 2.1 ORAL MICROBIOME

Humans, like all complex multicellular eukaryotes, are not autonomous organisms, but biological units which include numerous microbial symbionts and their genomes. The microbes in and on our bodies form a functional organ that is fundamental to our health and physiology. Together with our symbiotic microbial residents, they form a 'superorganism' or holobiont. The microbial component of the human holobiont is substantial, and at least equals the number of our own cells (Sender *et al*., 2016). The emergence of new genomic technologies, including next-generation sequencing (NGS) and bioinformatic tools, has provided a powerful means of understanding the contribution of the human microbiome to health (Kilian *et al*., 2016).

We have learned that we are not colonized at random, but that our microbial residents have coevolved with us over millions of years. The relationship between microbiome and host is dynamic, and influenced by many aspects of modern lifestyle, such as diet, tobacco consumption and stress, which can alter our microbiome and its properties, and induce a state in which this finely tuned ecosystem is no longer in balance. To address this divergence and maintain a harmonious state to protect health and prevent disease, we must not focus on the host and its residents as separate units, but, instead, consider the holobiont as one (Sender *et al*., 2016; Kilian *et al*., 2016).

## 2.2 BIOLOGICAL EVOLUTION

There is evidence that resident microbes have been performing metabolic functions in animals for at least 500 million years (Cho and Blaser, 2012). Coevolution is documented by the many similarities in the composition and organization of the human microbiome to that of other mammals (Blaser, 2006).

In humans, coevolution has also resulted in minor, although important, differences between ethnic groups (Haubek *et al*., 2008). The genetic material of microbes has followed us through our exodus from the birthplace of the human race in Africa, and has been used alongside human markers to trace migration routes across the planet (Rinaldi, 2007). In fact, detailed examination of strains of *Helicobacter pylori*

may allow us to distinguish human populations more accurately than a comparison of human genetic markers (Rinaldi, 2007). Throughout human evolution, our environment has continuously shaped the composition of our microbiome, increasingly so during the Neolithic, industrial revolution and modern eras (Gillings *et al*., 2015). The use of fire, the invention of agriculture, the increased access to processed foods, including refined sugar after the industrial revolution, and the advent of antimicrobial therapy, are all likely to have influenced the composition of the human microbiome (Gillings *et al*., 2015).

A study of calcified dental plaque samples from the time of transition from hunter-gather to Neolithic societies, and from the industrial revolution has proposed a compositional shift and declining microbial diversity around each of these evolutionary milestones (Adler *et al*., 2013). It is, however, reasonable to point out that there are limitations of microbiome determinations from ancient preserved samples compared with viable microbes sampled in modern days, and these findings must be interpreted with care. Introduction of refined sugar into our diet in the early times of agriculture caused certain oral bacteria to genetically evolve their metabolism, to adapt to 'post-agricultural' changes in our diet (Kilian *et al*., 2016).

In addition, since the industrial revolution, humans have been more frequently exposed to agents such as heavy metals, disinfectants, biocides and antibiotics that have the potential to eradicate or debilitate many microorganisms, while positively selecting those microbes that carry resistance determinants (Gillings *et al*., 2015). Oral hygiene practice changed towards the end of the nineteenth century in the developed world, mainly prompted by the publication of Willoughby Miller's book '*Microorganisms of the human mouth*' in 1890, which generated a worldwide promotion of teeth brushing and flossing (Kilian *et al*., 2016). This, too, is likely to have been a major factor in changes in the composition of the oral microbiome. The modern-day excessive consumption of acidic drinks and refined sugar or cigarette smoking has further impacted the oral ecosystem, leading to diseases such as cavities and periodontal disease (Kilian *et al*., 2016).

## 2.3 COMPLEX ECOLOGICAL COMMUNITY

It is known that the microorganisms (the microbiota) that make up the human microbiome are not just unicellular organisms living alongside each other, but instead form highly regulated, structurally and functionally organized communities attached to surfaces as biofilms, with interspecies collaborations as well as antagonisms that contribute to ecologic stability (Li and Tian, 2012). Bacteria within a biofilm can communicate with each other by producing, detecting and responding to small diffusible signal molecules in a process called quorum sensing, which brings benefits to host colonization, biofilm formation, defense against competitors and adaptation to changes in the environment (Li and Tian, 2012). Quorum-sensing activities in biofilms are also involved in the virulence and pathogenic potential of bacteria and are, therefore, an important factor in understanding and controlling bacterial infections, as they enable microorganisms in biofilms to become more tolerant of hosts' defenses and antimicrobial agents (Li and Tian, 2012).

The endogenous human microbial communities contribute to critical metabolic, physiological and immunological functions, including (Donohoe *et al.*, 2011):

- Differentiation and maturation of the host mucosa and its immune system
- Food digestion and nutrition
- Energy generation
- Metabolic regulation and control of fat storage
- Processing and detoxification of environmental chemicals
- Maintenance of skin and mucosa barrier function
- Development and regulation of the immune system and fine-tuning of its reaction pattern, that is, the balance between proinflammatory and anti-inflammatory processes
- Prevention of invasion and growth of disease-promoting microorganisms (colonization resistance).

Disruptions to the function and composition of the microbiome can have significant consequences for human health (Cho and Blaser, 2012). Despite variations in the composition of the microbiomes between individuals, it is important to note that the overall functions of their microbiota are relatively consistent (Gillings *et al*., 2015).

The most diverse bacterial populations are found in the gastrointestinal tract and the mouth (Kilian *et al*., 2016). The mouth is not a homogeneous environment for resident microbiota, but it offers several distinct habitats for microbial colonization, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, and hard and soft palate (Dewhirst *et al.,* 2010). These oral habitats form a highly heterogeneous ecological system and support the growth of significantly different microbial communities (Dewhirst *et al.,* 2010). The warm and moist environment in the mouth suits the growth of many microorganisms and offers host-derived nutrients, such as saliva proteins, glycoproteins and gingival crevicular fluid (GCF) (van 't Hof *et al.,* 2014). The teeth are the only natural non-shedding surfaces in the human body and provide unique opportunities for extensive biofilm formation, as well as safe haven for microbial persistence. Dental restorations, crown and bridgework, removable prostheses and implants constitute additional non-shedding surfaces in the mouth that can influence biofilm formation and composition (van 't Hof *et al.,* 2014).

To date, more than 700 prokaryotic taxa have been detected in the oral cavity, many of which cannot be isolated by common culture methods. Approximately 54% are validly named species, 14% are unnamed (but cultivated) and 32% are known only as uncultivated phylotypes (Kilian *et al*., 2016).

## 2.4 SYMBIOSIS ORAL MICROBIOME

During birth, the mother transmits microbes to the child, and delivery mode (vaginal *versus* caesarean) is, therefore, a determinant for the type of microorganisms that a child is initially exposed to (Dominguez-Bello *et al.,* 2010). Delivery mode also influences the diversity of the oral microbiome later on in an infant's life, with vaginally-born children showing a higher number of taxa 3 months after birth compared with children born by caesarean section (Lif *et al*., 2011). The method of feeding also has an effect, with 3-month-old breast-fed infants showing a higher colonization with oral lactobacilli than formulated infants (Lif *et al*., 2011). The eruption of teeth provides new surfaces for microbial colonization and constitutes a major ecological event in the mouth of a child (Sampaio-Maia and Monteiro-Silva, 2014). By the age of three, the oral microbiome of children is already complex, and becomes increasingly so with age. Replacement of the primary teeth with an adult dentition, again, significantly alters the oral microbial habitat (van 't Hof *et al.,* 2014).

Once established, the oral microbiome is maintained by host- and microbe-derived factors, involving processes that are still not fully understood. Resident bacteria have both pro- and anti-inflammatory activities that are crucial for maintaining homeostasis at heavily colonized sites, such as the oral cavity (Devine *et al*., 2015). Due to the interplay of the host's immune system with its microbial symbionts, acute infections of the oral mucosa are rather rare, despite dense microbial colonization (Zaura *et al*., 2014). The importance of these host-microbe interactions is highlighted by observations in immunosuppressed patients, who can experience life-threatening viral and fungal infections of the mucous membranes and oral infections by non-oral species (Devine *et al*., 2015; Zaura *et al*., 2014).

Both saliva and GCF provide nutrients for microbial growth and contain components with antimicrobial activities (van 't Hof *et al.,* 2014). The role of saliva in promoting oral health is well established (van 't Hof *et al.,* 2014). In addition to facilitating mastication, swallowing and speech, and aiding digestion, saliva contains vital enzymes and proteins that help maintain a balanced microbiota. Up to 108 microorganisms have been detected per milliliter of saliva, mostly derived from oral mucosal surfaces, such as the tongue (Marsh *et al*., 2016). Salivary components are the primary nutritional source for microorganisms, and are required for the development of a balanced microbiome. A large number of salivary components, including secretory immunoglobulin A, lactoferrin, lactoperoxidase, lysozyme, statherin and histatins, directly and indirectly regulate the microbiome, keeping it in balance (van 't Hof *et al.,* 2014). Another salivary component with antimicrobial potential is nitrite, converted from dietary nitrates by oral bacteria. Nitrite is further reduced to nitric oxide, which can inhibit growth of cariogenic bacteria, and thus may help to protect against caries (Doel *et al*., 2004).

Proteins, including enzymes, lipids and other components (carbohydrates, nucleic acids), mainly from saliva, but also derived from GCF, the oral mucosa and bacteria, form the acquired pellicle, which modulates attachment of bacteria to dental and epithelial surfaces and protects the tooth surfaces against acid attacks (Doel *et al*., 2004). Enzymes that help to regulate the balance of the microbiome are immobilized in the acquired pellicle in an active conformation. The individually composed acquired pellicle triggers and mediates bacterial adherence to the non-shedding tooth surfaces via various interactions (Doel *et al*., 2004).

Saliva not only helps to maintain an environment that allows biofilms to flourish, but also modulates the layers of plaque with the help of numerous proteins, including

enzymes and glycoproteins, as well as minerals, which control biofilm build-up and activity (van 't Hof *et al.,* 2014). Plaque biofilm is also dislodged by movement of the oral muscles of the cheeks and tongue during speech and mastication, and by the flow of saliva. The oral microbiota contributes to oral and general well-being, and its loss can be detrimental to the health of the individual.

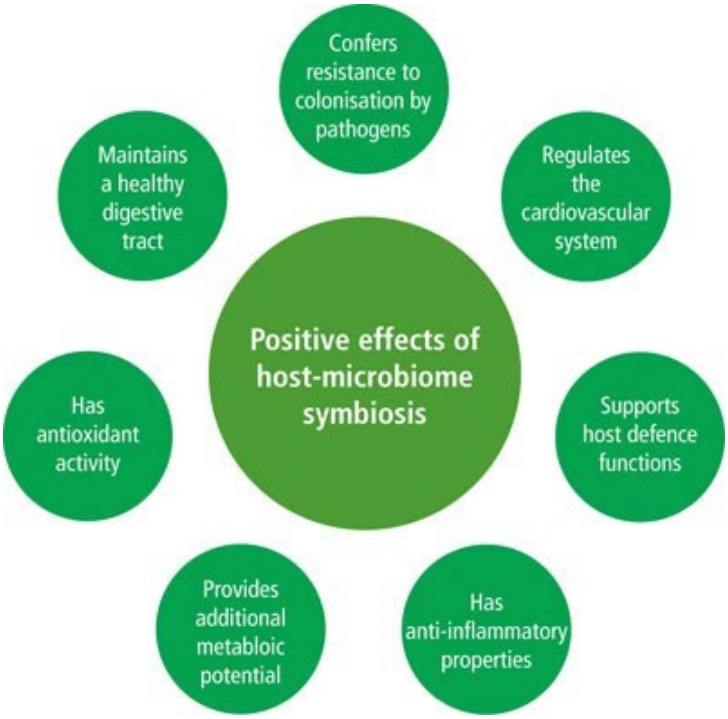


**Fig. 1 -** Positive effects of host-microbiome symbiosis (Kilian *et al.,* 2016).

## 2.5 DYSBIOSIS ORAL MICROBIOME

The complex equilibrium between resident species in the oral cavity is responsible for the maintenance of a healthy state (in symbiosis) or a state associated with disease (in dysbiosis). A dysbiotic microbiome is one in which the diversity and relative proportions of species or taxa within the microbiota is disturbed (Cho and Blaser, 2012). The relationship between the oral microbiome and its host is dynamic and, while in the healthy mouth the composition of microbial communities is remarkably stable (after the microbiome has matured in childhood), biological changes in a person's life can affect the balance of the species within these communities (Marsh *et al*., 2015). These include physiological changes, for instance, age, or hormonal changes in puberty and pregnancy, to which healthy individuals can often adapt without detriment to their oral health (Marsh *et al*., 2015). At other times, the finely-tuned ecosystem in the mouth can become disturbed, causing a dysbiotic shift and a loss of community balance or diversity in the biofilm, with a single or few species predominating, and an associated increased risk of disease. Modifiable factors driving oral dysbiosis include salivary gland dysfunction (that is, changes in saliva flow and/or composition), poor oral hygiene, gingival inflammation and lifestyle choices, including dietary habits and smoking (Marsh *et al*., 2014).

It is now an accepted concept that the bacteria historically considered as oral 'pathogens' can be found in low numbers at healthy sites, and oral disease occurs as a consequence of a deleterious change to the natural balance of the microbiota rather than as a result of exogenous 'infection' (Marsh *et al*., 2015). In dysbiosis, these disease-associated bacteria can grow to markedly higher proportions than under healthy conditions, where they are normally minor and innocuous components of the biofilm (Marsh *et al*., 2015).

Alterations in the pattern of biofilm formation may result in dysbiotic microenvironments in the many distinct habitats in the mouth. The distinct, non-shedding structure of teeth (smooth surfaces, pits and fissures, proximal sites and exposed root surfaces) enables large masses of microbes to accumulate as dental plaque biofilm (Marsh *et al*., 2014). Therefore, the plaque biofilm is not naturally shed as it accumulates, which is likely to be a key driver of dysbiosis in the absence of oral hygiene to disrupt and remove it. Oral bacteria have been proposed to play a role in a number of systemic diseases, including cardiovascular disease, rheumatoid arthritis, adverse pregnancy outcomes, stroke, inflammatory bowel disease and colorectal cancer, respiratory tract

infection, meningitis or brain abscesses, lung, liver or splenic abscesses, appendicitis, pneumonia and diabetes (Dewhirst *et al.,* 2010).

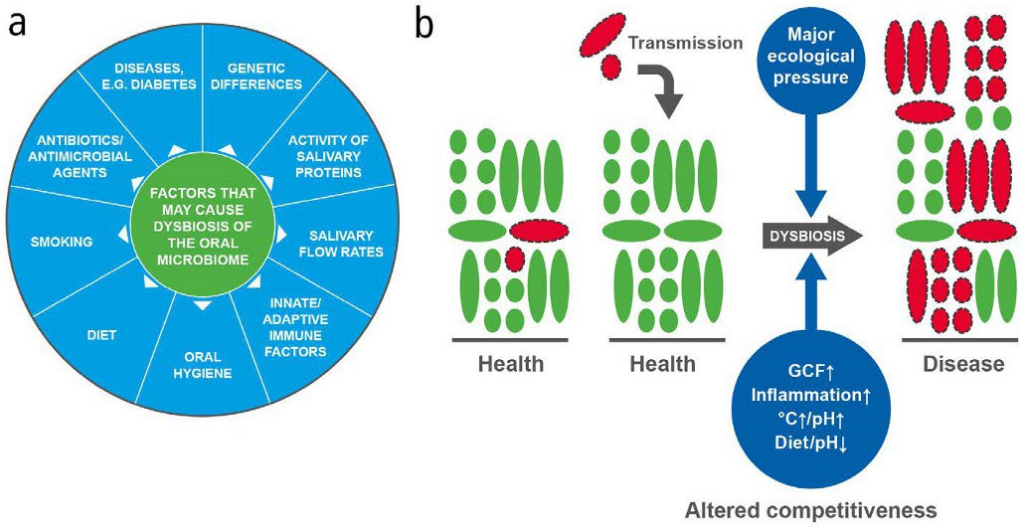


**Fig. 2 -** Causes of dysbiosis (a); A model of dysbiosis (b)

In health, the majority of the bacteria have a symbiotic relationship with the host; for the sake of simplification, these microorganisms are shown in green. Potentially cariogenic or periodontopathic bacteria (shown in red with dotted outlines) have been detected at healthy sites in low levels that are not clinically relevant; they may also be acquired from close partners (transmission), but again, their levels would be extremely low relative to the bacteria associated with health. In disease, there is an increase in the numbers and proportions of cariogenic or periodontopathic bacteria, and there may be increased biomass (especially in gingivitis). It is suggested that for this to happen, there has to be a change in local environmental conditions (major ecological pressure), which alters the competitiveness of bacteria within the biofilm and the selection of those species that are most adapted to the new environment. The factors driving this selection need to be recognised and addressed for adequate and consistent disease prevention (Kilian *et al.,* 2016).

## 2.6 PERI-IMPLANT DISEASE

Dental implants are medical–surgical devices placed in the jaw bones in order to replace one or more missing teeth with prosthetics (Butera *et al.,* 2022). The process that leads to integration of dental implants into the bone was described by Branemark in the 1960s and is called osseointegration, which is a direct connection, both structural and functional, between the vital bone and the surface of a loaded (i.e., prosthetic) implant (Butera *et al.,* 2022). Early implant loss takes place before prosthetic loading; in those cases, osseointegration is not successful, the implant is surrounded by connective tissue and can therefore not be used to anchor the planned prosthetic component (Korsch *et al*., 2021).

Peri-implant infections are the most common complications related to the placement of dental implants: they are classified into peri-implant mucositis and peri-implantitis (Rokaya *et al.*, 2020). According to the most recent guidelines, the diagnosis of peri-implant mucositis can be made if bleeding on probing (BOP), or suppuration is present in the absence of radiographic crestal bone loss (beyond initial remodeling). Peri-implantitis also involves bone resorption (beyond initial remodeling) and, consequently, an increase in probing pocket depth (PPD) (Berglundh *et al.,* 2018).

The prevalence of peri-implant mucositis and peri-implantitis can be as high as 80% and 56%, respectively (Rokaya *et al.*, 2020; Butera *et al.,* 2022).

Risk factors related to the development of peri-implant disease reported in the literature are: smoking, genetic factors such as a combined IL-1 genotype positivity, history of periodontitis, poor oral hygiene, systemic diseases (uncontrolled diabetes mellitus, cardiovascular and immunodepressive diseases), iatrogenic causes (such as extra cement), poor peri-implant soft tissue quality (keratinized gingiva thickness <2mm), history of one or more implant losses, excessive occlusal loading, and titanium particles (Butera *et al.,* 2022).

The primary etiological factor in the development of peri-implant diseases is the biofilm, which is a complex microbial community consisting of numerous micro-organisms that can communicate with each other through fine molecular processes (known as “quorum sensing”) (Huang *et al.,* 2011). The oral microbiota consists of more than 700 different species, which rarely live in planktonic form but aggregate in communities to form the biofilm; it can grow both on mineralized tooth surfaces, leading

to periodontitis, and on titanium implant surfaces, leading to peri-implant mucositis and, in the long term, peri-implantitis (Belibasakis *et al*., 2015; Huang *et al.,* 2011).

Salivary film, termed “acquired pellicle”, is a bacteria-free biofilm that covers dental and implant surfaces exposed to the oral cavity due to the presence of saliva; different surface receptors manifest in order to set up molecular links with late bacterial colonizers (Belibasakis *et al.*, 2015).

Salivary film on titanium surfaces does not include low molecular weight cystatins and mucins, in contrast with biofilms adherent to enamel surfaces. However, the underlying differences in the composition of films formed on titanium does not appear to represent a risk factor that can increase initial bacterial adhesion to implant surfaces (Costa *et al*., 2021).

It has been shown that only 30 minutes after implant insertion, there is a conspicuous bacterial colonization able to develop a well-organized biofilm in the peri-implant space after 2 weeks. In the following months, the peri-implant biofilm that has formed appears to be qualitatively less diversified in micro-organisms than that present on neighboring teeth, if present (Hu *et al.*, 2021).

There are many similarities in the microbial composition and immunological processes underlying the pathogenesis of periodontitis and peri-implantitis, but there are some significant differences which must be considered (Butera *et al.,* 2022).

From a histological and immunophysiological point of view, there are some important differences that make dental implants more susceptible to oral infections (Butera *et al.,* 2022). Whereas natural teeth are placed in the alveoli by the periodontal ligament (PDL), osseointegrated implants have a direct connection to the bone: the absence of the PDL reduces the blood flow to the supraperiosteal vessels and, consequently, limits the amount of nutrients and immunity cells that can come out of the vessels to deal with the ongoing bacterial infection (Belibasakis and Manoil, 2021). In addition, the arrangement of the supracrestal connective fibers is circumferential around the implants, rather than perpendicular as in natural teeth: this anatomical feature represents a less effective physical barrier against submucosal bacterial invasion (Belibasakis and Manoil, 2021).

## 2.7 PERI-IMPLANT MICROBIOLOGY

Implant health will be achieved if a symbiosis is established between the host and the peri-implant biofilm; however, in the presence of peri-implantitis risk factors, dysbiotic changes can occur to the microbiota constituting the peri-implant biofilm, setting off peri-implant soft tissue inflammatory processes, leading to peri-implant mucositis and peri-implantitis (Butera *et al.,* 2022). The implant material has gained interest in recent years as it may have a part to play in peri-implant biofilm dysbiosis (Nagay *et al*., 2022). As a result of the process of corrosion and attrition of the implant, caused by both the exposure of titanium to oral environment for long periods and the frictional forces developing physiologically at the implant-abutment interface, ions and nano- or microparticles of this metal may be released at the peri-implant soft tissue level (Nagay *et al*., 2022). To date, it is unclear whether such release of metallic material can establish a tissue inflammatory response and, in association with the presence of the microbial component, play an important role in the progression of peri-implant disease (Messous *et al.*, 2021).

Regarding the implant material, the addition of niobium and zirconium to the titanium implant alloy has been shown to have a similar bacterial adhesion pattern compared to implants composed of titanium and vanadium, with a slight increase in adhesion of *A. naeslundii* and *S. sanguinis* (Pantaroto *et al.*, 2019).

In the presence of poor oral hygiene for a period longer than three weeks, it has been found that dysbiosis of the peri-implant biofilm occurs, with bacterial proliferation of *Tannerella forsythia, Prevotella intermedia, Fretibacterium Fastidiosum* and *Treponema denticola* (Pantaroto *et al.*, 2019).

Although logic might suggest that implants and adjacent teeth have a similar microbiota because they share a similar ecological niche, i.e., the interdental space, more recent studies suggest the presence of important differences in diagnosis and therapy, probably due to different anatomy, histology, and peri-implant immunological characteristics (Zhang *et al.*, 2022).

The first studies aimed to identify bacteria around healthy implants and, in the presence of peri-implant pathologies, used anaerobic cultures and phase-contrast microscopy, detecting Gram-positive cocci and non-motile bacilli at the level of healthy implants. In the presence of peri-implant mucositis, a greater presence of cocci, motile

bacilli and spirochetes was observed, while other Gram-negative, motile and anaerobic species emerged in peri-implantitis (Belibasakis *et al*., 2015).

Subsequently, with the advent of newer techniques such as polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), or checkerboard DNA-DNA hybridization (CKB), a more precise inventory of micro-organisms involved in peri-implant infections has been provided, often assessing the presence of periodontopathogenic bacteria: this includes members of the “red complex” bacterial cluster, including *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*, but also *Treponema I-III* and *Synergistetes cluster A* (Belibasakis and Manoil, 2021).

From these early studies, the main differences in the peri-implant oral microbiota compared to the periodontal microbiota indicated the presence of pathogens, such as *Peptostreptococcus* spp. or *Staphylococcus epidermidis* and *Staphylococcus aureus* (Kensara *et al.*, 2021).

Through the advent of next-generation sequencing (NGS), which is a sequencing technology used to rapidly determine the order of nucleotides in whole genomes or targeted regions of DNA or RNA, it has been possible to provide quantitatively and qualitatively enhanced classification of the oral microbiota (Dewhirst *et al*., 2010).

In the first study which used NGS to compare the peri-implant and periodontal microbiota it was concluded that 85% of the individuals analyzed shared <8% bacteria between peri-implant and periodontal sites (Kumar *et al*., 2012). It was shown that the peri-implant microbiota appears to be, both in health and disease, quantitatively and qualitatively lower than the periodontal microbiota (Kumar *et al*., 2012). In addition, the authors highlighted the presence, at the peri-implant site, of bacterial genera that are not present at the periodontal site: for example, the genera Burkholderia, Anaerovorax, Anaerococcus, Aerofilium and Exiguobacterium. The predominant genera in the peri-implant microbiota were Butyrivibrio, Campylobacter, Eubacterium, Prevotella, Selenomonas, Streptococcus, Actinomyces, Leptotrichia, Propionibacterium, Peptococcus, Lactococcus, and Treponema. Implant sites with periimplantitis had lower concentrations of *Prevotella* and *Leptotrichia* and higher concentrations of *Actinomyces, Peptococcus, Campylobacter, Streptococcus nonmutans, Butyrivibrio, Pseudoramibacter alactolyticus,* and *Streptococcus mutans* than healthy peri-implant sites (Ghensi *et al.,* 2020; Kumar *et al*., 2012). Finally, this study found the presence of a higher amount of *Staphylococcus pettenkoferi* and *Staphylococcus hominis* in sites

with peri-implantitis compared to sites with periodontitis (Ghensi *et al*. 2020; Kumar *et al*., 2012).

In a later study, also based on the use of NGS, an increased concentration of *Prevotella nigrescens* was shown in sites with peri-implantitis, while bacteria such as *Peptostreptococcaceae* spp. and *Desulfomicrobium orale* were significantly higher in periodontitis. In addition, the greater the severity of peri-implantitis, the higher the concentration of *Treponema* sp. *HMT-257*, which is correlated with radiographic bone resorption, subsequent increase in peri-implant pocket, and suppuration (Maruyama *et al*., 2014).

Another study showed a gradual differentiation of the microbial community from peri-implant health to peri-implant mucositis and finally to peri-implantitis. An increased concentration of periodontal bacteria such as *Porphyromonas gingivalis, Tannerella forsythia*, and *Prevotella intermedia* was detected at sites with peri-implant mucositis, whereas in the presence of peri-implantitis, the study observed the existence of quantitatively rich microbial communities, with an increased concentration of bacteria from the genus Eubacterium spp. (Zheng *et al.*, 2015). Moreover, if the subject is a smoker, in healthy peri-implant sites the peri-implant microbiota is qualitatively less diversified, but there are more bacteria typical of peri-implant disease; instead, in sites with peri-implant mucositis, there is a quantitative reduction of bacterial species typically present in a healthy peri-implant site, also reducing its bacterial diversification; finally, it has been demonstrated that there are no qualitatively and quantitatively significant changes in the progression from peri-implant mucositis to peri-implantitis (Pimentel *et al*. 2018).

Bacteria from the classes Gammaproteobacteria (genus Vibrio), Epsilonproteobacteria (genus Campylobacter), and Bacilli (genus Granulicatella) were identified in greater amounts in the peri-implant crevicular fluid of healthy sites, whereas the classes Gammaproteobacteria (genus Acinetobacter and Moraxella) and Actinobacteria (genus Micrococcus) mainly appeared in sites with peri-implantitis (Gao *et al*., 2018).

Bacteria belonging to the genus Filifactor, typically found in sites with chronic periodontitis, Dialister, Mogibacterium, Propionibacterium, Acinetobacter, Staphylococcus, Paludibacter, and Bradyrhizobium were identified only at healthy peri-implant sites (Kotsakis and Olmedo, 2021).

The introduction of a new sequencing system, called MiSeq Illumina, has the advantage of reducing procedural error and increasing the ability to detect more bacterial species. It has been shown that, at healthy peri-implant sites, there is a predominance of bacteria belonging to the class Actinomycetia and bacterial species such as *Veillonella dispar, Rothia dentocariosa and Streptococcus sanguinis*, while in the presence of periimplantitis, the microbiota is characterized by the quantitative increase of bacteria belonging to the classes Bacteroidia, Spirochaetes, Synergistia (species *Synergistetes* spp. *HOT-360*), Clostridia (species *Clostridiales* spp. *HOT-093* and *Catonella morbi*), Deltaproteobacteria, of periodontopathogenic bacteria belonging to the "red complex" and finally of bacterial species such as *Porphyromonas* spp. *HOT-395*, *Porphyromonas nigrescens, Porphyromonas oris, Treponema maltophilum, Dialister invisus, Eubacterium saphenum, Filifactor alocis, Freitbacterium fastidiosum, Mitsuokella* spp. *HOT 131*, *Chloroflexi* spp., *Tenericutes* spp. and *Fretibacterium HMT 360* (Sanz-Martin *et al*., 2017).

Based on the studies in the literature, through the introduction of NGS and the MiSeq Illumina system, peri-implant and periodontal microbiota present quantitative and qualitative differences: in particular, the peri-implant microbiota presents less bacterial diversification than the periodontal microbiota, regardless of health or disease status, becoming more complex as it moves from peri-implant mucositis to periimplantitis (Zheng *et al.*, 2015). The microbial diversity detected between the peri-implant and periodontal microbiota should not be related to quantitative and qualitative changes in individual bacterial species, but rather to bacterial populations (Pimentel *et al*. 2018).

Results of the single study performed to date, based on the assessment of bacterial messenger RNAs (mRNAs), suggest that the intrinsic characteristics of the microorganisms composing the microbiota in sites with peri-implantitis and periodontitis, which favor the expression of bacterial pathogenicity, are similar to each other (Shiba *et al*., 2016). However, the capacity for inter-bacterial interaction appears to be more sophisticated at sites with peri-implantitis, with the presence of some significantly associated bacterial species (Shiba *et al*., 2016).

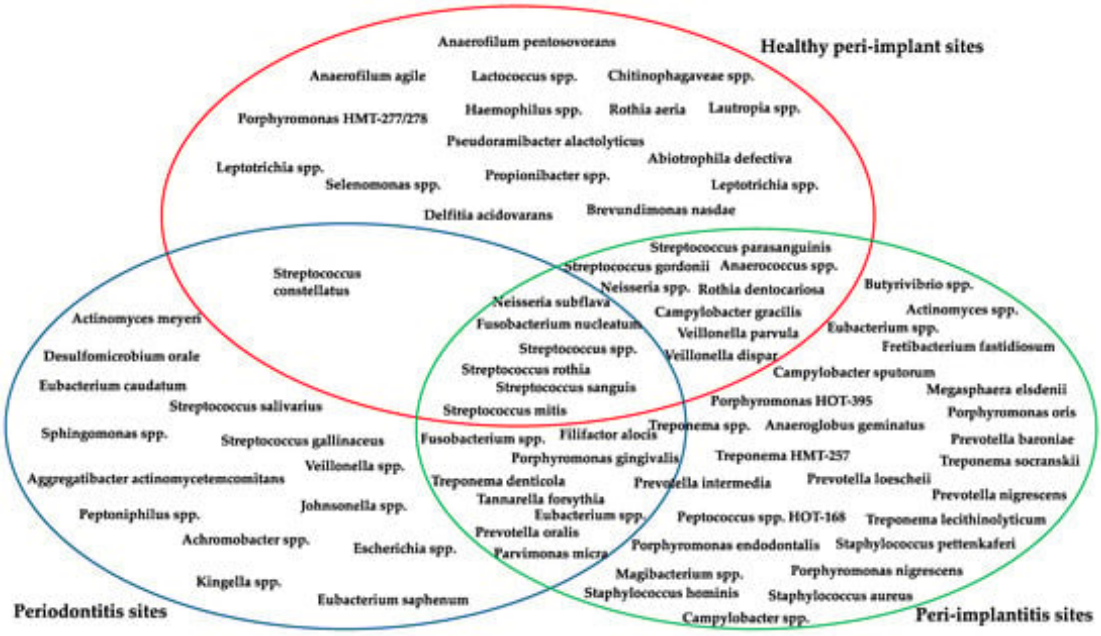


**Fig. 3 -** Bacterial species in healthy peri-implant sites and with peri-implantitis, in sites with periodontitis, and in common between the two sites (Butera *et al*., 2022).

## 2.8 ALVEOLAR BONE PRESERVATION

Loss of alveolar bone height can often be caused by preexisting periodontal disease. Smoking exacerbates loss of attached gingiva, gingival recession, and loss of alveolar bone height due to vasoconstriction in periodontal tissue by nicotine (Tatullo *et al*., 2016). In addition to periodontal disease, there are various factors associated with loss of alveolar bone height such as tooth extraction, tooth impaction or the presence of supernumerary tooth, oral injury, oral disease and oral surgery of jaw tumors (Daigo *et al*., 2020).

After eight weeks of healing, up to a 50% reduction of vertical bone wall height and a 20% horizontal bone resorption might be experienced by the patient. Over 12 months, 50% of the horizontal width of the ridge might disappear. Basically, the literature suggests that, within the first three months, almost two-thirds of bone reabsorption takes place (Schropp *et al*., 2003).

The difference in the position of blood clot formation in the extraction socket affects the height of new bone formation (Huebsch and Hansen, 1969). With the hemostatic methods typically used after tooth extraction (i.e., compression and suturing),

blood is not retained in the extraction socket, leading to considerable outflow of blood and eventual loss of alveolar bone height. In this way, worsened condition of alveolar bone leads to difficulty in occlusion and long-term maintenance of prosthetic function; which is the concept behind socket preservation (Daigo *et al*., 2020).

Bone deformities from tooth removal might be partially avoided and repaired by a procedure called socket preservation or alveolar ridge preservation (Horváth *et al*., 2013). Socket preservation consists of conservative procedures designed to maintain the volume of bone after the extraction. It helps counteract bone resorption and reduces the need for later bone augmentation, in anticipation of a fixed partial denture-pontic or implant placement. Therefore, socket preservation supports implant success and durability by minimizing bone resorption and increasing bone formation (Horváth *et al*., 2013).

Currently, autologous bone grafting, using a bone filler or a collagen sponge, is performed for socket preservation. This procedure effectively preserves the blood clot in the extraction socket and offers coverage with osteoanagenesis-inducing substances. However, favorable outcomes are not necessarily achieved, probably due to infected granulation tissue and chronic residual inflammation in the socket, infection after bone or artificial bone grafting, and incomplete substitution of bone with synthetic material (Brawn and Kwong-Hing, 2007; Cordaro *et al*., 2012). Thus, thorough curettage of the socket, sufficient bleeding from the socket wall, and good preservation of the blood clot are essential. Laser irradiation to hasten wound healing and achieve reliable retention of blood in the extraction socket is reported to minimize alveolar bone resorption in the clinical setting (Brawn and Kwong-Hing, 2007; Cordaro *et al*., 2012).

## 2.9 BONE REGENERATION

Regeneration of bone tissue after oral and maxillofacial surgery reconstruction remains a challenge in medicine and dentistry. Bone defects are the main reason for aesthetic and functional disability and negatively affect a patient's quality of life (Amaroli *et al*., 2020). A primary objective after bone tissue surgery is to restore the natural morphology and function of the impacted region (Sakkas *et al*., 2017). Bone and bone-substitute grafts are, in different ways, the gold standards for bone grafting, due to their peculiar characteristics in containing bone matrix proteins and osteogenic cells, which support bone growth (Sakkas *et al*., 2017). However, they might show postoperative

complications, which are seen in at least 30,000 patients per year, worldwide. In the dentistry field, dental implants are more commonly accepted tools, and thousands of implants are placed every year by specialists and general practitioners. However, more than 10% of bone surgeries and related procedures can have healing complications as a consequence of infections, tissue damage, or inadequate blood supply and cell energy default, which lead to the alveolar bone reabsorption after tooth extraction (Amaroli *et al*., 2020).

Different patterns of bone resorption might occur after tooth extraction. It is difficult to predict the final ridge contour and dimensions due to the remodeling of alveolar tissues, which considerably affects oral rehabilitation with dental implants and other prosthetic tools (Amaroli *et al*., 2020). The socket healing process might be conceptualized as a sequence of biological steps occurring after tooth extraction, to fill the dental alveolus with bone tissue (Schropp *et al*., 2003). Essentially, following a tooth extraction, a defensive fight against infection occurs via polymorphonucleocyte cell migration and coagulum formation in the impacted area. Fast angiogenesis takes place and it is accompanied and followed by an osteoclastic activity, which carries out the bone breakdown to create gaps within the bone and to promote step-by-step remodeling (Araújo and Lindhe, 2005).

Many techniques, materials and complementary therapies have been described in recent years to improve alveolar preservation for extraction site grafting. A number of graft materials could be used (Ellegaard, 1976):

1. Autogenous grafts: Grafts transferred from one position to another within the same individual. This type of graft comprises cortical bone or cancellous bone and marrow, and is harvested either from intraoral or extraoral donor sites;

2. Allogeneic grafts: Grafts transferred between genetically dissimilar members of the same species. Frozen cancellous bone and marrow and freeze-dried bone are used;

3. Xenogenic grafts: Grafts taken from a donor of another species;

4. Alloplastic materials: Synthetic or inorganic implant materials that are used as substitutes for bone grafts.

The action of the grafting material used could be differentiated between (Ellegaard, 1976):

1. Osteoproliferative action (osteogenetic): New bone is formed by bone-forming cells (osteoblasts) contained in the grafted material; this is typical of autogenous grafts;

2. Osteoconductive action: The grafted material does not contribute to new bone formation per se, but serves as a scaffold for bone formation originating from the adjacent host bone; this happens with xenogenic and alloplastic grafts;

3. Osteoinductive action: Bone formation is induced in the surrounding soft tissue immediately adjacent to the grafted material; the most widely studied type of osteoinductive cell mediator is the bone morphogenetic protein (BMP) family.

The clinician, therefore, has a wide variety of graft materials with distinct properties that could be used for the clinical procedure; it is up to the clinician to choose the best material for the right case. Basically, socket preservation is done to preserve the alveolar space derived from the tooth extraction. The main aim is to keep the space in the alveolar socket and prevent the collapse and resorption of the bone all around (Amaroli *et al*., 2020).

## 2.10 IMMEDIATE IMPLANTS

Dental implants are currently accepted as a predictable treatment option for the rehabilitation of both partial or total edentulism. Moreover, immediate and early loading protocols have been introduced into clinical practice in the attempt to shorten treatment time and minimize patient discomfort, with positive results (Baldi *et al.,* 2018). During the early stages of healing, dental implants should be protected from detrimental micromovements which, according to the literature, should not exceed values ranging between 50 and 150$\mu$m to avoid risks for the osseointegration process (Szmukler-Moncler *et al*., 1998). When exceeding this threshold, there is a concrete possibility that the bone-implant interface could be colonized by fibroblasts from the overlying connective tissue, with consequent implant encapsulation in fibrous tissue and clinical failure. In this scenario, the role of primary stability has become extremely important and, in recent years, many studies have focused on this crucial topic (Raghavendra *et al.,* 2005).

Immediate placement of a dental implant in an extraction socket was initially described more than 40 years ago by Schulte and Heimke in 1976 (Ortega-Martínez *et al*., 2012; Schulte and Heimke, 1976).

Reductions in the number of surgical interventions, shorter treatment time, an ideal three-dimensional implant positioning, the presumptive preservation of alveolar bone at the site of the tooth extraction and soft tissue aesthetics have been claimed as the potential advantages of this treatment approach (Chen *et al.,* 2004).

On the other hand, the morphology of the side, the presence of periapical pathology, the absence of keratinized tissue, thin tissue biotype and lack of complete soft tissue closure over the extraction socket have been reported to adversely affect immediately placed implants (Chen *et al.,* 2004).

The first classification described the timing of implant placement as mature, recent, delayed or immediate depending on soft tissue healing and predictability of guided bone regeneration (GBR) procedures. However, further classifications based on hard and soft tissue healing and treatment time approach were subsequently described, as shown in Table 1 (Hämmerle *et al*., 2004; Esposito *et al*., 2006).

The efficacy of GBR therapy employing autogenous and non-autogenous particulate materials combined with various membranes to regenerate alveolar bone at the time of tooth extraction has also been demonstrated. Concomitant placement of regenerative materials has been shown to result in predictable, high levels of osseointegration (Hämmerle *et al*., 2004; Esposito *et al*., 2006).

| Author / Year | Classification | Implant Placement |
|---|---|---|
| **Hämmerle *et al*. (2004)** | Type I | In fresh extraction sockets |
| | Type II | After soft tissue coverage (4-8 weeks) |
| | Type III | Radiographic bone fill (12-16 weeks) |
| | Type IV | Healed socket (> 16 weeks) |
| **Esposito *et al*. (2006)** | Immediate | In fresh extraction sockets |
| | Immediate-delayed | < 8 weeks post extraction |
| | Delayed | > 8 weeks post extraction |

**Table 1 -** Time of implant placement (Ortega-Martínez *et al*., 2012).

Quirynen *et al*. (2007) focused their review on immediate versus delayed implant placement. Most papers contained only data on implant loss, but did not provide useful information on implant failure or hard and soft tissue changes.

Despite many articles describing limited marginal bone level or gain in immediate implant therapy, caution is needed as few of these studies report radiographic outcomes (Ortega-Martínez *et al*., 2012).

Several reviews reported that the immediate implant treatment using autogenous bone grafts or xenografts may improve the process of bone formation between the implant and the surrounding socket walls, as well as survival rates (Chen *et al*., 2004; Fugazzotto, 2005).

If the gap jumping distance between the socket wall and the implant is over 2 mm, it has been reported that grafting is recommended, while smaller distances could heal spontaneously (Chen *et al*., 2004; Fugazzotto, 2005).

Post-extraction implants have survival rates similar to implants placed on healed sites; nevertheless, some guidelines could be drawn (Ortega-Martínez *et al*., 2012):

- Interproximal bone level and soft tissue recession: Crestal bone as well as soft tissue preservation could be achieved with either immediate implant placement following tooth extraction or a delayed protocol;

- Treatment of the gap between implant and bone wall: There is no consensus whether bone augmentation with GBR at immediate implants placed into fresh extraction sites is necessary, and which is the most predictable procedure;

- Presence of periapical infection: Chronic periapical infection is a risk factor but not an absolute contraindication for immediate implant placement. However, debridement of the alveolus should be made. The presence of a periapical infection should be carefully weighed;

- Primary implant stability: Is an important factor in achieving osseointegration. Several methods have been used to quantify this parameter, such as insertion torque values and resonance frequency analysis (RFA). The presence of sufficient primary implant stability, together with other factors like minimally traumatic surgical technique and implant macro and microgeometry, are considered crucial factors to obtaining and maintaining implant osseointegration (Menini *et al*., 2017). However, while these general concepts are currently widely accepted, it is more challenging to define and control the

different variables influencing the achievement of adequate primary stability (Baldi *et al*., 2018).

As suggested by McCullough and Klokkevold, implant macrogeometry plays a fundamental role: variations in implant length, diameter, number of threads, thread depth, pitch, and helix angle may strongly influence primary stability.

It must also be considered that excessive compression of the host bone, caused by high insertion torques, could result in a prolonged inflammatory phase: even if inflammation is always the necessary basis for tissue repair, massive and long-lasting presence of proinflammatory cytokines could result in delayed healing and marginal bone resorption (Teixeira *et al*., 2015). Moreover, high insertion torques could cause permanent deformations of the implant platform (especially external hex connections), possibly jeopardizing long-term maintenance and stability of the entire prosthetic rehabilitation (Teixeira *et al*., 2015).

## 2.11 LASER LIGHT-OSTEOBLAST INTERACTION

The light–cell interaction is well-known in plant cells, where during the first phase of chlorophyll photosynthesis (light phase), solar energy is absorbed by chlorophyll and other pigments located in the membranes of thylakoids, inside the chloroplasts (Amaroli *et al.*, 2020). However, light–cell interactions are also described in non-plant cells, such as non-photosynthesizing prokaryotic and protozoan cells as well as animal cells (Amaroli *et al.*, 2019). When a photon interacts with a specific photoacceptor, its energy is absorbed to generate high-energy electrons. The excited molecule can shed its energetic status in the form of heat or fluorescence emission, or the absorbed light energy can be transferred to a photosystem molecule as an excited electron or state. In this way, the photosystem converts the photon's energy into chemical energy, thanks to the complex process of electron transport and a proton gradient, ending with the conversion of ADP into ATP (Amaroli *et al.*, 2020). In plants, this process occurs in the chloroplast, whereas it occurs in photoacceptors in bacteria and the conversion of ADP takes place in the inner part of the cell membrane. In other eukaryotic cells, electron transport occurs in the mitochondrial respiratory chain. This close interconnection between chloroplasts and mitochondria reflects their common origin through their bacterial ancestors and the parallel and convergent evolution of endosymbiont models (Stefano *et al*. 2015).

Particularly, Fe-protoporphyrin (heme), Fe–S clusters, and chromophore proteins with $Cu^{2+}$ centers of complex IV in the mitochondrial inner membrane respiratory chain show suitable features to be photoacceptors. Evidence based on the literature points out that cytochrome c oxidase (complex IV) exhibits evident absorption peaks at the red (600–700nm) and near-infrared (NIR) (760–900nm) wavelengths (according to its precise oxidation state); complexes I and II are not affected by light in this spectrum, while at 808 nm, complex III is poorly stimulated (Amaroli *et al*., 2016). However, by increasing the wavelength to 1064 nm, the photon and mitochondrial complex interaction changes and, while the I, III, IV, and V complexes are affected, the extrinsic mitochondrial membrane complex II and mitochondrial matrix enzymes seem to not be receptive to photons at this wavelength (Ravera *et al*., 2019). It is, however, of interest to take into account that other photoacceptors can exist and be involved in the ATP-, ROS-, and calcium-dependent cellular pathways, following red and IR light stimulation, such as water, transient receptor potential-V cation channels, and cellular membranes (Amaroli *et al*., 2016; Amaroli *et al*., 2019; Ravera *et al*., 2019). Consequently, photons can affect

animal cell behavior; this medical subject heading is defined as Photobiomodulation (PBM), previously known as low-level laser therapy (Amaroli *et al.*, 2020).

However, the mechanisms through which PBM works are multifaceted and are involved in versatile biological actions such as gene expression, energy metabolism, cell proliferation, differentiation, survival, and cell death (Amaroli *et al*., 2016).

## 2.12 LASER BONE PRESERVATION

Alveolar bone and soft tissue remodeling are a normal physiological response following tooth extraction (Križaj *et al*., 2021; MacBeth *et al*., 2017). The resorption process varies amongst patients and tooth position and may be affected by several factors, such as the presence of infection, previous periodontal disease, the extent of a traumatic injury, and the number or thickness of the bony socket walls (MacBeth *et al*., 2017).

The complex alveolar post-extraction repair process is marked by cellular and molecular events such as vessel growth, cell proliferation, differentiation, and the synthesis and the release of cytokines and growth factors (Rosero *et al*., 2020). Briefly, the alveolar bone repair begins with blood clot characterizing the inflammatory phase, which will gradually be replaced by granulation tissue consisting of neovascular tissues, inflammatory cells, and erythrocytes, which will produce collagen fibers and will start the mineralization process, resulting in the immature bone. Then, the immature bone will be progressively remodeled into trabecular and spongy bone filling the alveolar socket (Park *et al*., 2015; Rosero *et al*., 2020).

The newly grown/generated bone will undergo remodeling, an inevitable and irreversible physiological phenomenon that results in alterations in alveolar bone height and thickness (Hämmerle *et al*., 2012). Also, there is a plethora of factors that strike bone metabolism like the presence of metabolic diseases, aging, smoking, and local trauma that may negatively affect the remodeling process resulting in an extensive bone loss (Klokkevold and Han, 2007; Rosero *et al*., 2020).

An equilibrium is reached approximately 3 to 4 months post-extraction (MacBeth *et al*., 2017). The clinical consequences of post extraction remodeling may affect the outcome of the ensuing therapies aimed at restoring the lost dentition, either by limiting the bone availability for ideal implant placement or by compromising the aesthetic result

of the prosthetic restorations (Kulkarni *et al.,* 2018). Therefore, effective methods of reducing bone loss, accelerating bone healing, and making it more predictable are actively sought. Most studies focus on drugs or surgical techniques although more recently other modalities affecting the healing process have been investigated; among which is the use of laser therapy (Noba *et al*., 2018).

PBM is probably the best researched use of lasers in post extraction healing (Kulkarni *et al.,* 2018; Noba *et al*., 2018). Recent reviews of accumulated animal and clinical studies reported that laser PBM therapy induced higher concentration of osteogenesis markers, as well as higher bone density and concluded that PBM improved the post-extraction healing process. However, the results vary with laser wavelength and parameters used (Križaj *et al*., 2021; Kulkarni *et al*., 2018; Noba *et al*., 2018).

Blood clot is very important for proper uncomplicated socket healing (Križaj *et al*., 2021). Laser irradiation of bleeding sockets may facilitate immediate clot formation and hemostasis (Aoki *et al*., 2015). Different types of lasers and diodes have been used successfully to coagulate blood and prevent the loss of blood clot from extraction sockets, resulting in improved alveolar bone preservation (Aoki *et al*., 2015). Bactericidal effect of laser therapy is considered advantageous for postoperative wound healing because lasers are capable of creating a disinfected field during surgery and reducing the risk of infection (Aoki *et al*., 2015). In addition, because the neodymium-doped yttrium aluminum garnet (Nd:YAG) laser exhibits selective absorption in pigments, it is conceivable that this laser would be effective for devitalizing some of the pigmented bacteria, such as *Porphyromonas gingivalis*, that are associated with periodontal disease (Aoki *et al*., 2015). This aspect may be particularly relevant for extractions performed due to periodontal disease. Moreover, lasers can ablate or inactivate toxic substances, such as bacterial endotoxins (lipopolysaccharide), which may positively influence wound healing of the treated site and offer several advantages over conventional mechanical treatment (Aoki *et al*., 2015).

PBM with Nd:YAG laser has been found to improve healing after extraction in patients at high risk for osteonecrosis (Vescovi *et al*., 2015). Use of erbium-doped yttrium aluminum garnet (Er:YAG) laser for degranulation has been studied in periodontal and peri-implant treatment and seemed to promote osseointegration on contaminated implant surfaces to a greater degree than alternative methods (Aoki *et al*., 2015). The advantages of laser degranulation are improved hemostasis and disinfection and Er:YAG laser may be safely used because its high absorption in water results in very

efficient ablation with minimal thermal effect. This property of the Er:YAG laser also allows for very fine control of depth of ablation, which makes it highly suitable for fast and safe de-epithelialization of the gingiva surrounding the extraction socket (Grzech-Leśniak *et al.*, 2018). This de-epithelialization prevents ingrowth of epithelium into the socket and, at the same time, produces an ablated rough surface, which may enhance retention of the blood clot (Aoki *et al*., 2015).

Laser post extraction procedure consisting of degranulation, disinfection, de-epithelialization, clot stabilization and photobiomodulation using Er:YAG and Nd:YAG lasers significantly improves bone healing at 4 months post-extraction (Križaj *et al*., 2021).

The Use of barrier membranes has significant positive effects on the outcomes of alveolar ridge preservation, indicating that clot stabilization and prevention of epithelial ingrowth are important contributing factors in the final result (Bassir *et al*., 2018).

The results obtained with Nd:YAG PBM are more consistently positive, possibly due to great penetration depth of this wavelength. Nd:YAG laser irradiation after tooth extraction promotes osteoblast differentiation, as demonstrated by the higher expression of osteocalcin in experiments in rats (Mergoni *et al*., 2016).

# CHAPTER 3 – MATERIALS AND METHODS

## APPENDIX - 1

## CLINICAL PROTOCOL

# 3.1 CLINICAL PROTOCOL

## Università Cattolica del Sacro Cuore di Roma

**The effect of laser treatment on alveolar bone preservation and immediate implant placement - randomized controlled trial**

### 3.1.1 INTRODUCTION

The placement of osseointegrated dental implants is a reliable treatment option for partially and totally rehabilitating edentulous patients. Despite high success rates, the individual optimization of treatment protocols is crucial for prognosis, patients' satisfaction and analysis of potential risk factors for dental implant failure. Over an observation period of 10 years, a survival rate of 85–95% can be estimated (Al-Nawas *et al*., 2012). In 5%, the absence of primary implant integration results in implant failure (Le Guéhennec *et al*., 2007) and an intra-individual accumulation of implant losses might imply the existence of specific risk factors for dental implant failure (DIF) (Peled *et al*., 2003; Weyant and Burt, 1993).

DIF can be subdivided into early and late events: early DIF is associated with impaired bone healing (namely insufficient bone-implant contact and fibrous scar formation) (Le Guéhennec *et al*., 2007; Mohajerani *et al*., 2017) while Late DIF occurs after a latency period of 6 months (Mohajerani *et al*., 2017).

Risk factors can be subdivided into iatrogenic, material-associated and patient-related factors (van Steenberghe *et al*., 2003). Side effects during surgery include heat-induced necrosis, poor primary stability, and incorrect positioning (Alsaadi *et al.,* 2007; el Askary *et al*., 1999a; el Askary *et al*., 1999b). The geometry of the implants - including the implant dimensions and its macro design, as well as the type of prosthetic connection, affects the load distribution and, consequently, the survival rate of dental implants. Local risk factors include significant plaque accumulation, gingivitis, tight implant-tooth contact, bone quality and quantity, poor oral hygiene, periodontal disorders, and chronic occlusal trauma (Alsaadi *et al.,* 2007). In addition, systemic factors such as xerostomia, osteoporosis, cardiovascular disease and diabetes mellitus are reported to influence patients' wound healing capability (Mohajerani *et al*., 2017; van Steenberghe *et al*., 2003).

### 3.1.2 LASER THERAPY

Alveolar bone and soft tissue remodeling are a normal physiological response following tooth extraction (Križaj *et al*., 2021; MacBeth *et al*., 2017). The resorption process varies amongst patients and tooth anatomic position and may be affected by several factors such as the presence of infection, previous periodontal disease, the extent of a traumatic injury and the number or the thickness of the bony socket walls (Križaj *et al*., 2021; MacBeth *et al*., 2017). An equilibrium is reached approximately 3 to 4 months post-extraction (Križaj *et al*., 2021; MacBeth *et al*., 2017). The clinical consequences of post-extraction remodeling may affect the outcome of the ensuing therapies aimed at restoring the lost dentition, either by limiting the bone availability for ideal implant placement or by compromising the aesthetic result of the prosthetic restorations (Kulkarni *et al*., 2018). Therefore, effective methods of reducing bone loss, accelerating bone healing, and increasing predictability are actively sought. Most studies focus on drugs or surgical techniques although other modalities affecting the healing process have been investigated; among which is the use of laser therapy (Noba *et al*., 2018).

Photobiomodulation (PBM) in post extraction healing is well documented; accumulated animal and clinical studies reported that PBM laser therapy induced higher concentration of osteogenesis markers and higher bone density (Kulkarni *et al*., 2018; Lemes *et al*., 2019).

The laser wavelength and parameters used are of crucial importance; Nd:YAG laser (1064nm) has been found to improve healing after extraction in patients with high risk of osteonecrosis (Mohajerani *et al*., 2017). The Er:YAG laser (2940nm) for degranulation has been studied in periodontal and peri-implant treatments. It seems to promote re-osseointegration on contaminated implant surfaces, and improve haemostasis and disinfection (van Steenberghe *et al*., 2003).

The Er:YAG laser may be safely used in hard and soft tissues due to its high absorption in water, resulting in efficient ablation with minimal thermal effect. This feature of the Er:YAG laser also allows very fine control of ablation depth, which makes it highly suitable for fast and safe de-epithelialization of the extraction socket and the surrounding gingiva (Alsaadi *et al.,* 2007). This de-epithelialization prevents ingrowth of epithelium into the socket and at the same time, produces an ablated rough surface, which may enhance retention of the blood clot (van Steenberghe *et al*., 2003).

Laser irradiation of bleeding sockets may facilitate immediate clot formation and hemostasis (Aoki *et al*., 2015; Križaj *et al*., 2021). Different types of lasers have been used successfully in coagulation to prevent the loss of blood clot from extraction sockets in animal studies, resulting in improved alveolar bone preservation (Aoki *et al*., 2015; Križaj *et al*., 2021). The Bactericidal effect of laser therapy is considered advantageous for postoperative wound healing once lasers are able to create an intra-operative disinfected field that reduces the risk of infection (Aoki *et al*., 2015; Križaj *et al*., 2021). In addition, Nd:YAG laser exhibits selective absorption in pigments, that may be particularly relevant for extractions performed due to periodontal disease. Moreover, lasers can ablate or inactivate toxic substances, such as bacterial endotoxins (lipopolysaccharide), which may positively influence wound healing of the treated site (Aoki *et al*., 2015; Križaj *et al*., 2021).

### 3.1.3 HYPOTHESIS

The aim of this study is to objectively evaluate a comprehensive post-extraction with immediate implant placement laser protocol versus post-extraction with immediate implant placement standard protocol.

This laser protocol consists of degranulation, disinfection, decortication, de-epithelialization, clot stabilization and photobiomodulation using Er:YAG and Nd:YAG wavelengths.

The results will evaluate the comparison between measurements of alveolar bone loss and bone density with cone-beam computed tomography (CBCT), implants insertion torque (IT), implants resonance frequency analysis (RFA) and clinical side effects in each group.

### 3.1.4 PATIENTS

Participants for this randomized clinical trial were recruited among patients attending the combined implantology/oral surgery outpatient clinic of Clitrofa – Centro Médico, Dentário e Cirúrgico Lda; in Trofa - Portugal, between July 2022 and February 2023. Inclusion criteria encompass patients of either sex, aged 18-80 years, in whom simple or multiple teeth extractions are indicated, who agreed to participate in the study and signed informed consent. Exclusion criteria are pregnancy, recent antibiotic use,

smoking, uncontrolled diabetes or high blood pressure, use of photosensitizing medication and medications or conditions that would compromise bone healing. The total number of implants for this study is 50. Extractions and immediate implant placement procedure will be randomized into laser and control groups (1:1) through the drawing of closed envelopes.

In the control group, the tooth is extracted, the socket is mechanically curetted, and the implant is placed immediately. In the laser group, the procedure is the same, however, additionally, before implant placement, the laser protocol is applied in the socket.

This study will be led according to the World Medical Association declaration of Helsinki – ethical principles for medical research involving human subjects.

### 3.1.5 PROCEDURE

Initial diagnosis will be based on intraoral dental examination and CBCT imaging analysis. Each patient's age, sex, teeth number, location, and indication for extraction will be recorded. Local anesthetic (Lidocaine/ Epinephrine 20 mg/ml + 0.0125 mg/ml solution for injection EFG) will be infiltrated before extraction and implant placement in both groups.

In the control group the standard post-extraction procedure will be carried out with cleaning of the post-extraction socket with a surgical curette. In the laser group, Er:YAG and Nd:YAG lasers (LightWalker®, Fotona®, Slovenia) will be used immediately after tooth extraction with the following protocol:

-Step 1: **Degranulation** - Er:YAG handpiece H14 with cylindrical sapphire tip 1.3mm diameter, 160mJ per pulse Energy, 15Hz Frequency, 2.4W average Power, Water/Air: 4/2, SP Mode;

-Step 2: **Disinfection** - Nd:YAG handpiece 300µm fiber with non-contact, 20Hz Frequency, 2.0W average Power, 100mJ per pulse Energy, 10 seconds per wall, SP Mode;

-Step 3: **Decortication** - Er:YAG handpiece H14 with cylindrical sapphire tip 1.3mm diameter, 300mJ per pulse Energy, 15Hz Frequency, 4.50W average Power, Water/Air: 5/2, QSP Mode;

-Step 4: **De-Epithelialization** - Er:YAG handpiece H14 with cylindrical sapphire tip 1.3mm diameter, 40mJ per pulse Energy, 30Hz Frequency, 1.20W average Power, Water/Air: 1/2, SP Mode;

-Step 5: **Clot Stabilization** - Nd:YAG handpiece 300µm fiber with non-contact, 30Hz Frequency, 4.0W average Power, 0 J per pulse Energy, 60 seconds, VLP Mode;

-Implant placement - The implants used will be Epikut® line, double acid etching (DAA), with cone morse prosthetic connection (S.I.N.-Implant System®, Brazil).

-Step 6: **Photobiomodulation (LLLT)** - Nd:YAG Genova handpiece, 1cm² spot size, MSP mode, 10Hz Frequency, 0.5W average Power, 60 seconds oral and 60 seconds vestibular, performed on the day of extraction and day 1, 3, 4, 6 and 8. All laser group patients included in the analysis received at least 4 of the 5 scheduled photobiomodulation sessions.

Antibiotic (amoxicillin 875mg + clavulanic acid 125mg for 8 days) for patients not allergic to penicillin and Anti-inflammatory analgesic (100mg nimesulide for 8 days) was prescribed.

**Fig. 4 -** LightWalker® laser from Fotona®.


**Fig. 5 -** The 6 steps LightWalker® laser settings protocol. Step 1: Degranulation using Er:YAG; Step 2: Disinfection with Nd:YAG; Step 3: Decortication using Er:YAG; Step 4: De-Epithelialization with Er:YAG; Step 5: Clot Stabilization using Nd:YAG and Step 6: Photobiomodulation (LLLT) with Nd:YAG.

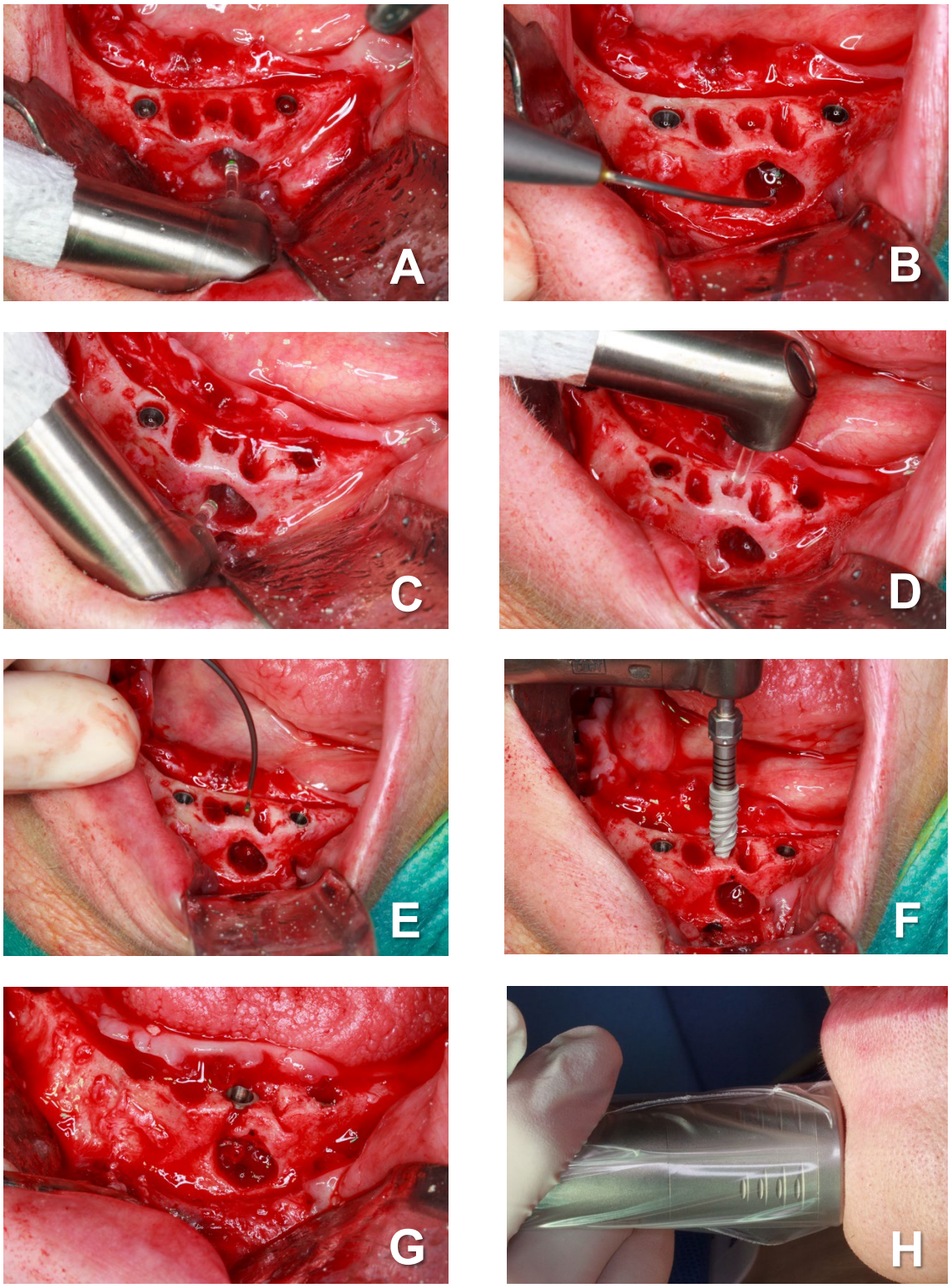


**Fig. 6 -** Clinical application sequence of the 6 steps LightWalker® laser protocol. A: Degranulation using Er:YAG; B: Disinfection with Nd:YAG; C: Decortication using Er:YAG; D: De-Epithelialization with Er:YAG; E: Clot Stabilization using Nd:YAG; F: Immediate implant placement; G: Clinical aspect of the implant and H: Photobiomodulation (LLLT) with Nd:YAG.

### 3.1.6 OUTCOME MEASURES

1) CBCT Scans: Blinded evaluation of bone volume and density from CBCT (NewTom 5G®, Italy), this equipment uses Safebeam$^{TM}$ technology that automatically adjusts the radiation dose according to the patient's age and size. This technology uses intermittent bursts of radiation, which last only milliseconds, during image acquisition. The scan obtains a complete dentomaxillofacial image in a single database of digital information.

The following parameters were used: X-ray source - 110KV, 1-20mA (pulsed mode); focal spot - 0.3mm; acquisition technique - single scan; scan time 18-36 seconds' exposure; X-ray emission time 3.6s-6.7s; signal grey-scale - 14 bit scanning and 16-bit reconstruction; FOV size DxH - 6x6 centimeters; patient positioning - supine;

Measurements were performed at two different times, namely on the day of extraction and implant placement and after 4 months (MacBeth *et al*., 2017).

NewTom NNT Analysis software (NewTom®, Italy) was used to plot the bone and implants on CBCT scans. Using this software, the three-dimensional information from the post-operative CBCT image was compared with the pre-operative CBCT image and the bone loss and bone density evaluated.

2) Insertion Torque (IT) Measurement: The maximum IT value of each implant was recorded with SurgicPro 2 (NSK®, Japan). The IT will automatically increase in single unit increments until the operator was unable to rotate the implant due to friction, before complete insertion of the implant.

Measurements were performed at two different times, namely on the day of extraction and implant placement and after 4 months (MacBeth *et al*., 2017).

The insertion torque is aimed to fall within 30–50 Ncm and may be adjusted by using a larger implant or rotating the implant in the opposite direction if the value is out of the range.

3) Resonance Frequency Analysis: Evaluation of resonance frequency analysis (RFA) was performed using the Osseo 100 device (NSK®, Japan). Measurements were performed at three different times, namely immediately after implant placement, 2

months after surgery and 4 months after surgery. The SmartPegs should be mounted on the implants and manually screw-tightened. The RFA value was measured twice in each of the three measurements. RFA values are represented in the unit called the implant stability quotient (ISQ), which ranges from 1 to 100. A higher ISQ value indicates greater stability (Sennerby and Meredith, 2008).

4) Potential side effects: Pain rating was measured on a scale from 0 to 10 (0-no pain to 10-unspeakable pain). Measurements were performed at three different times, namely during treatment, 8 days after surgery and 30 after surgery. Other complications such as bleeding, swelling, trismus, implant failure and bone loss were also monitored.

### 3.1.7 STATISTICAL ANALYSIS

IBM® SPSS® statistics software, version 28, was used for both descriptive and inferential data analysis. First, the study variables were tested to ensure they conformed to a normal distribution by using either the Kolmogorov-Smirnov's ($D$) or the Shapiro-Wilk's ($W$) test. Secondly, the variances of the samples were tested for homogeneity by using either the Levene's ($L$) or the Bartlett's ($B$) test.

Descriptive measures included the arithmetic mean ($\bar{x}$), the standard deviation ($SD$), and the standard error of the mean ($SE$), as well as the 95% confidence interval ($95\%\ CI$). Where the data were not normally distributed, the mode, the frequencies, the median and the inter-quartile range ($IQR$) were used.

In those situations where the data were normally distributed and the variances were constant, a comparative analysis between the control and experimental groups was made by the unpaired two-tailed Student's ($t$) test. The comparative analysis between the pre-test and post-test measures for each one of the study groups (control and experimental) was made by the paired two-tailed Student's ($t$) test.

Where the data were not normally distributed and/or the variances were not constant, the following non-parametric inferential tests were used: Mann-Whitney ($U$) test for comparison of the control and experimental groups; Wilcoxon Signed Rank ($U$) test for comparison of pre-test and post-test measures for each one of the study groups (control or experimental).

The following diagram is hereby presented for purposes of clarification:

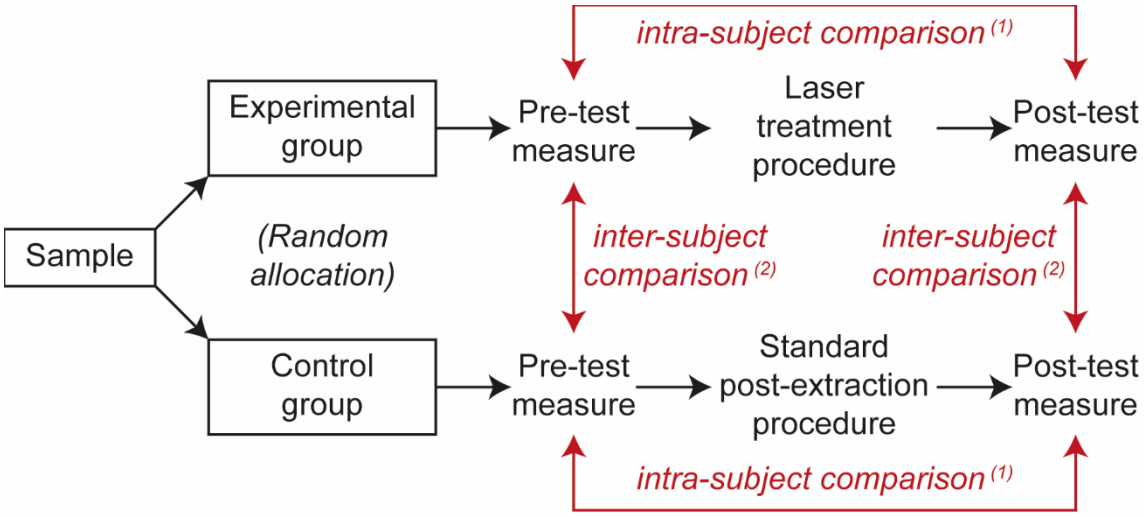


**Fig. 7 -** Statistical Analysis diagram. (1) Paired-Samples Student's (t) test or Wilcoxon Signed Rank's (U) test; (2) Unpaired-Samples Student's (t) test or Mann-Whitney's (U) test.

The minimum level of significance ($\alpha$ level) accepted throughout the development studies was 0.05 ($*$), considered to be moderately significant. Levels of 0.01 ($**$) were considered as significant and 0.001 ($***$) designated as highly significant. A lack of statistical significance was designated as ($ns$).

# CHAPTER 3 – MATERIALS AND METHODS

## APPENDIX - 2

## PATIENT INFORMATION

## 3.2 PATIENT INFORMATION SHEET

## Università Cattolica del Sacro Cuore di Roma

Please read this form carefully. Please ask if you do not understand or would like more information.

### GENERAL INFORMATION GUIDELINES

Title of research project: The effect of laser treatment on alveolar bone preservation and immediate implant placement - randomized controlled trial

Name of Investigator: Dr. Fernando Duarte
Supervisors: Prof. Ilay Maden and Prof. Giovanni Olivi

We would like your help in a study designed to allow us to decide which of two surgical approaches for treating patients with immediate implants after tooth extraction is the best. It will involve you being randomly allocated to one of two groups.

Teeth replacement using dental implants has proven to be a successful and predictable treatment procedure; different placement and loading protocols have evolved from the first protocols, in order to achieve quicker and easier surgical treatment times.

Immediate placement of a dental implant in an extraction socket is a common procedure. Reductions in the number of surgical interventions, shorter treatment time, an ideal three-dimensional implant positioning, the presumptive preservation of alveolar bone at the site of the tooth extraction and soft tissue aesthetics have been claimed as the potential advantages of this treatment approach.

In order to find out which of these two approaches is the best, we need to do this study. You will be assigned to one of the two treatment groups after your initial investigation has been done. In the control group, the tooth is extracted, the socket is mechanically curetted, and the implant is placed immediately. In the laser group, the procedure is the same, however, additionally, before implant placement, the laser protocol is applied in the socket. There is no difference between the two techniques in terms of post-operative pain, swelling, or recovery time.

During your recovery period you will be followed up in our out-patient department, and your progress will be monitored with clinical observations and X-rays. The post-operative follow-up is the same whether or not you agree to take part in the study.

If you do not wish to participate in this study you are free to refuse to do so and it will not affect your care.

Your personal information will be treated as confidential and kept secure.

You will be kept informed of all relevant facts arising as the project progresses.

The expected duration of the study is 4 months.
Participation in this project does not involve any restriction on your activities or drug administration.

You may ask questions to the investigator on any matters relating to your participation in the proposed research project.

# CHAPTER 3 – MATERIALS AND METHODS

## APPENDIX - 3

## INFORMED CONSENT FORM

## 3.3 CONSENT FORM FOR RESEARCH ON PATIENTS

### Università Cattolica del Sacro Cuore di Roma

Please read this form carefully and ask if you do not understand or would like more information.

### CONSENT BY THE PATIENT

Title of Research: The effect of laser treatment on alveolar bone preservation and immediate implant placement - randomized controlled trial

Name of Investigator: Dr Fernando Duarte

I……...(Full name) of

……(Address), hereby fully and freely consent to participating in the above-mentioned research project.

I agree that my general practitioner may be notified of my participation in the research project and that they may release information on my past history. I have informed the investigator of any drugs I am presently taking.

I understand and acknowledge that the investigation is designed to promote medical knowledge.

I understand that I may withdraw my consent at any stage in the investigation.

I acknowledge the purpose of the investigation, the nature and purpose of which has been detailed to me during a personal interview and has been explained to me by:

**Dr Fernando Duarte**

Signed…….. Date……

**DECLARATION BY THE INVESTIGATOR**

I confirm that I have informed the above-named patient during a personal interview and explained the nature and effect of the procedures so that their consent has been given freely and voluntarily.

Signed…….

Name……

# CHAPTER 3 – MATERIALS AND METHODS

## APPENDIX - 4

## PATIENT RECORDING DATA

## 3.4 PATIENT RECORDING DATA

### Università Cattolica del Sacro Cuore di Roma

**Name of Subject:** ……

**D.O.B:** ……/……/……

**Sex:** ☐ Female ☐ Male

**Group:** ☐ Control ☐ Laser

**Medical History:**

☐ Smoking ☐ Non-Smoking

☐ Light (< 10 cigarettes/day)

☐ Heavy (≥ 10 cigarettes/day)

☐ Diabetes

☐ Hypertension

☐ Osteoporosis

**Reason for Extraction:**

☐ Chronic periodontitis

☐ Periapical granuloma

☐ Vertical root fracture

☐ Horizontal root fracture

☐ Deep decay

☐ Trauma

**Number of Extractions per patient:**

☐ 1
☐ 2
☐ 3
☐ 4
☐ 5
☐ 6
☐ 7
☐ 8
☐ 9
☐ 10
☐ 11
☐ 12
☐ 13
☐ 14

**Extraction site/ Implant placement:**

☐ Maxilla
☐ Mandible

**Tooth extracted / Implant placement:**

☐ Incisor
☐ Canine
☐ Premolar
☐ Molar

**Implant Dimensions:**

| Code DAA | Diameter (mm) | Length (mm) |
|---|---|---|
| ☐ ILCM 3585 | 3,5 | 8,5 |
| ☐ ILCM 3510 | 3,5 | 10 |
| ☐ ILCM 3511 | 3,5 | 11,5 |
| ☐ ILCM 3513 | 3,5 | 13 |
| ☐ ILCM 3515 | 3,5 | 15 |
| ☐ ILCM 3885 | 3,8 | 8,5 |
| ☐ ILCM 3810 | 3,8 | 10 |
| ☐ ILCM 3811 | 3,8 | 11,5 |
| ☐ ILCM 3813 | 3,8 | 13 |
| ☐ ILCM 3815 | 3,8 | 15 |
| ☐ ILCM 4585 | 4,5 | 8,5 |
| ☐ ILCM 4510 | 4,5 | 10 |
| ☐ ILCM 4511 | 4,5 | 11,5 |
| ☐ ILCM 4513 | 4,5 | 13 |
| ☐ ILCM 4515 | 4,5 | 15 |
| ☐ ILCM 5085 | 5 | 8,5 |
| ☐ ILCM 5010 | 5 | 10 |
| ☐ ILCM 5011 | 5 | 11,5 |
| ☐ ILCM 5013 | 5 | 13 |
| ☐ ILCM 5015 | 5 | 15 |

**Insertion Torque Measurement:** ……NCm

**Resonance Frequency Measurements:** ……ISQ

☐ Immediately after implant placement

First Measurement ……ISQ  Second Measurement ……ISQ

☐ 2 months after surgery

First Measurement ……ISQ  Second Measurement ……ISQ

☐ 4 months after surgery

First Measurement ……ISQ  Second Measurement ……ISQ

**Pain Scale:**

☐ During Treatment

☐ 1, ☐ 2, ☐ 3, ☐ 4, ☐ 5, ☐ 6, ☐ 7, ☐ 8, ☐ 9, ☐ 10

☐ 8 days after surgery

☐ 1, ☐ 2, ☐ 3, ☐ 4, ☐ 5, ☐ 6, ☐ 7, ☐ 8, ☐ 9, ☐ 10

☐ 30 days after surgery

☐ 1, ☐ 2, ☐ 3, ☐ 4, ☐ 5, ☐ 6, ☐ 7, ☐ 8, ☐ 9, ☐ 10

**Complications:**  ☐ No  ☐ Yes

☐ Bleeding  ☐ Swelling  ☐ Trismus  ☐ Implant Failure  ☐ Bone Loss

# CHAPTER 4 – RESULTS

## 4.1 PATIENT GENERAL INFORMATION

Fourteen patients attending the combined oral surgery/implantology clinic at the Clitrofa – Centro Médico, Dentário e Cirúrgico, in Trofa - Portugal, scheduled for tooth extraction and immediate implant placement at the same surgical time, had their data recorded in the following categories: sociodemographic, clinic, and reason for tooth extraction.

Table II summarizes the main characteristics of the participants of the study, including the control group and the experimental (laser) group.

| | Control Group | Experimental (Laser) Group | Total |
|---|---|---|---|
| **Sociodemographic** | | | |
| **Number of participants (N)** | 8 | 6 | 14 |
| **Age (Mean ± SD)** | 48.13 ± 14.97 | 57.17 ± 13.45 | 52.00 ± 14.52 |
| **Sex (Frequencies)** | Females: 6 (75.0%)<br>Males: 2 (25.0%) | Females: 2 (33.3%)<br>Males: 4 (66.6%) | Males: 8 (57.1%)<br>Females: 6 (42.9%) |
| **Clinic** | | | |
| **Number of implants (N)** | 25 | 25 | 50 |
| **Extractions (Mean ± SD)** | 4.88 ± 5.10 | 5.50 ± 5.24 | 5.14 ± 5.14 |
| **Smoking (Frequencies)** | Non-Smoker: 7 (87.5%)<br>Heavy Smoker: 1 (12.5%) | Non-Smoker: 5 (83.3%)<br>Heavy Smoker: 1 (16.7%) | Non-Smoker: 12 (85.7%)<br>Heavy Smoker: 2 (14.3%) |
| **Diabetes (Frequencies)** | Non-Diabetic: 8 (100.0%)<br>Diabetic: 0 (0.0%) | Non-Diabetic: 5 (83.3%)<br>Diabetic: 1 (16.7%) | Non-Diabetic: 13 (92.9%)<br>Diabetic: 1 (7.1%) |
| **Hypertension (Frequencies)** | Non-Hypertense: 8 (100.0%)<br>Hypertense: 0 (0.0%) | Non-Hypertense: 5 (83.3%)<br>Hypertense: 1 (16.7%) | Non-Hypertense: 13 (92.9%)<br>Hypertense: 1 (7.1%) |
| **Osteoporosis (Frequencies)** | Non-Osteoporotic: 8 (100.0%)<br>Osteoporotic: 0 (0.0%) | Non-Osteoporotic: 6 (100.0%)<br>Osteoporotic: 0 (0.0%) | Non-Osteoporotic: 14 (100.0%)<br>Osteoporotic: 0 (0.0%) |
| **Reason for Extraction** | | | |
| **Chronic Periodontitis (Freq.)** | No: 5 (62.5%)<br>Yes: 3 (37.5%) | No: 3 (50.0%)<br>Yes: 3 (50.0%) | No: 8 (57.1%)<br>Yes: 6 (42.9%) |
| **Periapical Granuloma (Freq.)** | No: 8 (100.0%)<br>Yes: 0 (0.0%) | No: 4 (66.7%)<br>Yes: 2 (33.3%) | No: 12 (85.7%)<br>Yes: 2 (14.3%) |

| | | | |
|---|---|---|---|
| **Vertical Root Fracture (Freq.)** | No: 8 (100.0%)<br>Yes: 0 (0.0%) | No: 6 (100.0%)<br>Yes: 0 (0.0%) | No: 14 (100.0%)<br>Yes: 0 (0.0%) |
| **Horizontal Root Fracture (Freq.)** | No: 8 (100.0%)<br>Yes: 0 (0.0%) | No: 5 (83.3%)<br>Yes: 1 (16.7%) | No: 13 (92.9%)<br>Yes: 1 (7.1%) |
| **Deep Decay (Frequencies)** | No: 4 (50.0%)<br>Yes: 4 (50.0%) | No: 6 (100.0%)<br>Yes: 0 (0.0%) | No: 10 (71.4%)<br>Yes: 4 (28.6%) |
| **Trauma (Frequencies)** | No: 7 (87.5%)<br>Yes: 1 (12.5%) | No: 6 (100.0%)<br>Yes: 0 (0.0%) | No: 13 (92.9%)<br>Yes: 1 (7.1%) |

**Table 2 -** Sociodemographic and clinical characterization of the sample.

No statistically significant differences have been found between the control and experimental groups regarding the sociodemographic and clinical variables depicted in table I ($p > 0.05$), except for the prevalence of deep decay ($p = 0.043$).

Table II depicts the anatomical position and model of the implants placed, as well as the patient identification number and research group (control and experimental).

| Implant Position | Implant Model | Patient | Group |
|---|---|---|---|
| **2.5 Maxilla** | 4515DAA | 001 | Laser |
| **2.1 Maxilla** | 4513DAA | 002 | Laser |
| **2.2 Maxilla** | 4513DAA | 002 | Laser |
| **1.1 Maxilla** | 4511DAA | 003 | Control |
| **3.1 Mandible** | 3513DAA | 004 | Laser |
| **3.4 Mandible** | 3513DAA | 004 | Laser |
| **4.2 Mandible** | 3513DAA | 004 | Laser |
| **4.4 Mandible** | 3513DAA | 004 | Laser |
| **2.6 Maxilla** | 4510DAA | 005 | Laser |
| **1.1 Maxilla** | 4585DAA | 006 | Laser |
| **1.4 Maxilla** | 4511DAA | 006 | Laser |
| **1.6 Maxilla** | 4510DAA | 006 | Laser |
| **2.1 Maxilla** | 4585DAA | 006 | Laser |
| **2.4 Maxilla** | 4511DAA | 006 | Laser |
| **2.6 Maxilla** | 4510DAA | 006 | Laser |
| **1.5 Maxilla** | 4510DAA | 007 | Control |
| **3.1 Mandible** | 4515DAA | 008 | Control |
| **3.4 Mandible** | 5010DAA | 008 | Control |
| **3.6 Mandible** | 5010DAA | 008 | Control |
| **4.1 Mandible** | 4515DAA | 008 | Control |
| **4.4 Mandible** | 5010DAA | 008 | Control |
| **4.6 Mandible** | 5010DAA | 008 | Control |
| **1.2 Maxilla** | 5015DAA | 009 | Control |
| **1.6 Maxilla** | 5015DAA | 009 | Control |
| **2.2 Maxilla** | 5015DAA | 009 | Control |
| **2.6 Maxilla** | 5015DAA | 009 | Control |

| | | | |
|---|---|---|---|
| **1.2 Maxilla** | 3815DAA | 010 | Control |
| **1.5 Maxilla** | 3810DAA | 010 | Control |
| **2.2 Maxilla** | 3815DAA | 010 | Control |
| **2.5 Maxilla** | 3810DAA | 010 | Control |
| **1.2 Maxilla** | 3511DAA | 011 | Laser |
| **1.4 Maxilla** | 3811DAA | 011 | Laser |
| **1.6 Maxilla** | 4585DAA | 011 | Laser |
| **2.2 Maxilla** | 3811DAA | 011 | Laser |
| **2.4 Maxilla** | 3813DAA | 011 | Laser |
| **2.6 Maxilla** | 4510DAA | 011 | Laser |
| **3.2 Mandible** | 3513DAA | 011 | Laser |
| **3.4 Mandible** | 3811DAA | 011 | Laser |
| **3.6 Mandible** | 3510DAA | 011 | Laser |
| **4.2 Mandible** | 3513DAA | 011 | Laser |
| **4.4 Mandible** | 3811DAA | 011 | Laser |
| **2.6 Maxilla** | 4510DAA | 012 | Control |
| **3.6 Mandible** | 4515DAA | 013 | Control |
| **1.1 Maxilla** | 3513DAA | 014 | Control |
| **1.3 Maxilla** | 4511DAA | 014 | Control |
| **1.5 Maxilla** | 4511DAA | 014 | Control |
| **2.1 Maxilla** | 3513DAA | 014 | Control |
| **2.3 Maxilla** | 4511DAA | 014 | Control |
| **2.4 Maxilla** | 5013DAA | 014 | Control |
| **2.7 Maxilla** | 4511DAA | 014 | Control |

**Table 3 -** Anatomical position, model of the implant, patient identification number and research group of the patient.

## 4.2 OUTCOME 1 - CBCT SCANS

### 4.2.1 BONE DENSITY

The term “bone quality” encompasses many broad concepts of bone, including physiology, mineralization, and morphology (Kinalski *et al*., 2021). According to the classification suggested by Lekholm and Zarb, bone density can be classified into four types based on the amount of cortical versus cancellous bone in the alveolar bone examined on pantograph film (Sarkis-Onofre *et al*., 2019). Misch further characterized the four bone density classes based on the tactile sense of the clinician placing the implant. However, a distinction between the four types of bone has not been clearly established (Kim *et al*., 2021).

CBCT is useful when assessing the relative distribution of compact and cancellous bone. It has been reported that 1mm thick cross-sectional images provide

more accurate bone height measurements than 2-mm thick (or thicker) cross-sectional images, thus displaying the anatomic region of interest based on thicker sections may hamper the diagnosis due to superposition of adjacent structures (Nikolic-Jakoba *et al*., 2021).

Bone density can be evaluated using Hounsfield units (HU), which are expressed by computer tomography attenuation values according to a linear density scale (Shapurian *et al*., 2006). The Hounsfield scale is used to evaluate bone for implant placement, and these values were considered site specific, objective and quantitative. HU value is related to the density of the tissue represented by the voxel bone density classification, which is categorized as follows: D1, >1250 HU; D2, 850–1250 HU; D3, 350–850 HU; D4, 150–350 HU (Sogo *et al*., 2012).

The poorest intraoral bone quality is typically found in the posterior maxilla, more than 80% of the edentulous posterior maxillae consists of porous cortical crest or no cortical bone (Shapurian *et al*., 2006). Although most of the posterior maxillae were classified as D3 or D4, there were remarkable variations among individuals (Shapurian *et al*., 2006).

Generally, bone quality is considered the primary cause of different survival rates examined in the maxilla and mandible. Modifications in implant design, implant number, and surgical techniques are required to better suit implant surgery in D4 bone (Kim and Lim, 2011). A study of 3937 patients who had received a total of 12,465 dental implants, reported implant survival rates according to bone density of: type I, 97.6%; type II, 96.2%; type III, 96.5%; and type IV, 88.8% (Goiato *et al*., 2014).

At the largest length scales, two types of bone structure are evidenced: trabecular and cortical bone. Trabecular or cancellous bone micro-architecture shows a porous network with small inter-woven filaments, which results in higher porosity (80%-85%) comparing to cortical area (2-5%) (Irie *et al*., 2018).

Bone quality involves bone mass, structural properties: geometry, macro and micro-architecture, and tissue properties: modulus of elasticity, mineral density, collagen quality, cell and marrow behavior. Mechanical and biological behavior of bone tissue play an important role in clinical practice, especially for evaluating distinct systemic and local conditions (diseases, therapies or lesions) (Irie *et al*., 2018).

The peri-implant marginal bone and its alterations are another very important and reliable outcome in regard to implant success and survival (Kinalski *et al*., 2021;

Papaspyridakos *et al*., 2012). It has been reported that a successful implant would not have a marginal bone loss greater than 1.5 mm in the first year, and in the subsequent years, it should be restricted to 0.2 mm per year (Kinalski *et al*., 2021; Papaspyridakos *et al.*, 2012).

CBCT is increasingly replacing multislice computed tomography (MSCT) for evaluating mineralized tissues, because it provides adequate image quality associated with a lower exposure dose. Other advantages of CBCT are its low cost, fast scanning time and lower number of image artifacts (Silva *et al*., 2012).

### 4.2.2 NEWTOM 5G

The geometric accuracy for linear measurements with CBCT is high, so that bone dimensions and implant proximity to relevant normal anatomic structures can be accurately assessed (Lou *et al*., 2007; Worthington *et al*., 2010).

The radiation dose from any CBCT device largely depends on the type of the machine and scan settings, including field of view (FOV), number of basis projections and scan modes, among other factors (Parsa *et al*., 2013; Pauwels *et al*., 2012).

Similar to radiation dose, the influence of FOV and scan settings on image quality is significant. Within any CBCT system, image quality itself is inconsistent and also largely dependent on the selected FOV and scan settings (Mah *et al.*, 2010). Depending on the number and location of the potential implant to be placed, the size of the chosen FOV will differ. To date, the influence of FOV and other scan setting selections can have on grey value measurements obtained from CBCT remains unverified (Parsa *et al*., 2013).

CBCT is the modality of choice for pre-operative dental implant surgery assessment and post-operative diagnostic evaluation (Parsa *et al*., 2013).

The NewTom 5G cone-beam CT scanner, produced by NewTom (Italy) feature the NNT software, a proprietary software that creates different kinds of 3D images, compatible with third party software. With a high-resolution flat panel detector, 16-bit dynamic range, a powerful X-ray source with a very small focal spot (0.3mm) and a rotating anode, 5G produces the sharpest image possible with today's technology. The size of selectable FOV can vary from 6x6cm to 18x16cm, which can be chosen depending on the particular clinical application. NewTom optimizes the use of radiation via its unique SafeBeam™ technology: X-rays are pulsed only in sync with the acquisition

of raw images. In this way, the dose that is actually absorbed is less than with a comparable exam with a conventional MSCT.

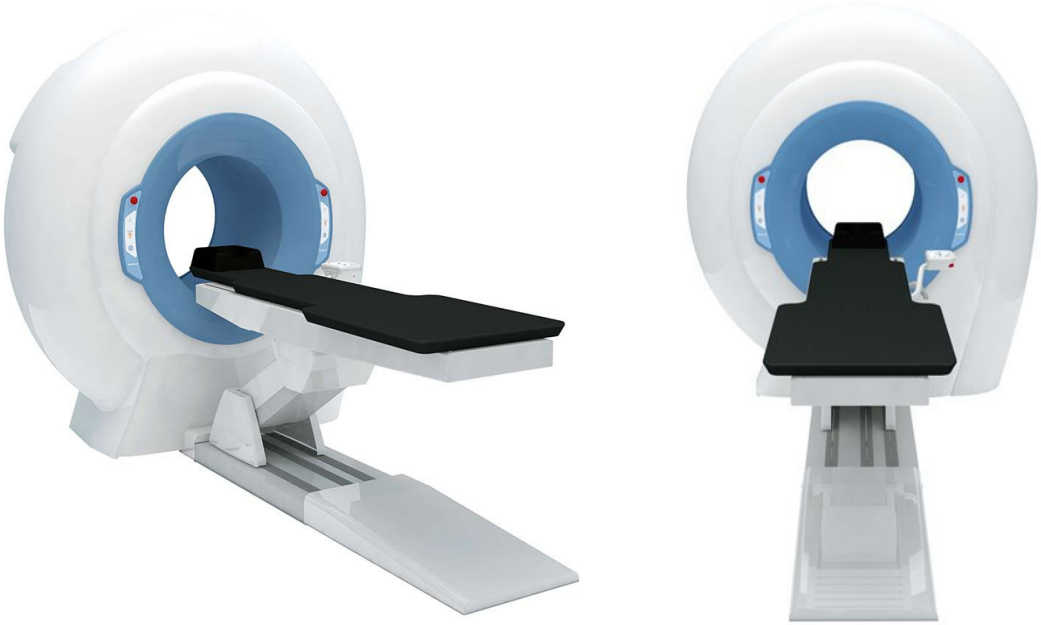

**Fig. 8 -** NewTom 5G CBCT Machine.

### 4.2.3 PILOT STUDY 1 PROTOCOL

Fourteen patients attending the combined oral surgery/implantology clinic at the Clitrofa – Centro Médico, Dentário e Cirúrgico, in Trofa - Portugal, scheduled for tooth extraction and immediate implant placement at the same surgical time, were tested according to the following protocol:

CBCT X-ray source - 110KV, 1-20mA (pulsed mode); focal spot - 0.3mm; aquisition technique - single scan; scan time 18-36 seconds exposure; X-ray emission time 3.6s-6.7s; signal grey-scale - 14-bit scanning and 16-bit reconstruction; FOV size DxH - 6x6 centimeters; patient positioning - supine;

Measurements were performed at two different times, namely on the day of extraction and implant placement and after 4 months (MacBeth *et al.*, 2017).

NewTom NNT Analysis software (NewTom, Italy) was used to plot the bone and implants on CBCT scans. Using this software, the three-dimensional information from the post-operative CBCT image was compared with the 4-month follow-up CBCT image; bone height and bone density were evaluated.

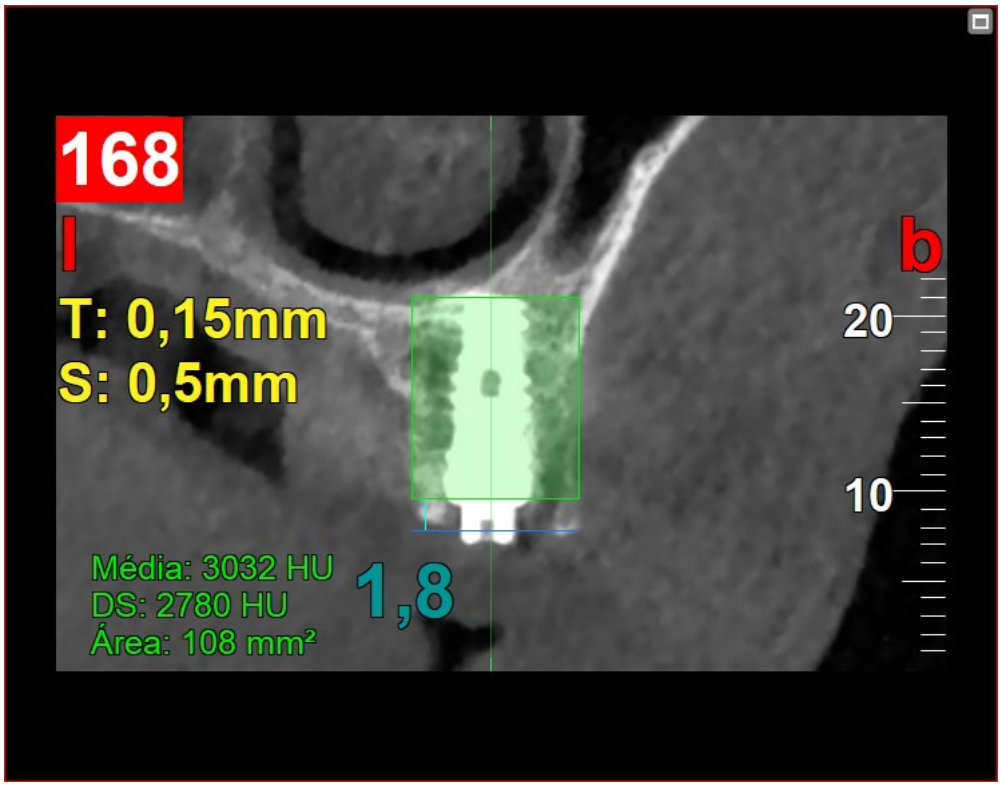


**Fig. 9 -** Immediate post-operative CBCT considering bone height and density.

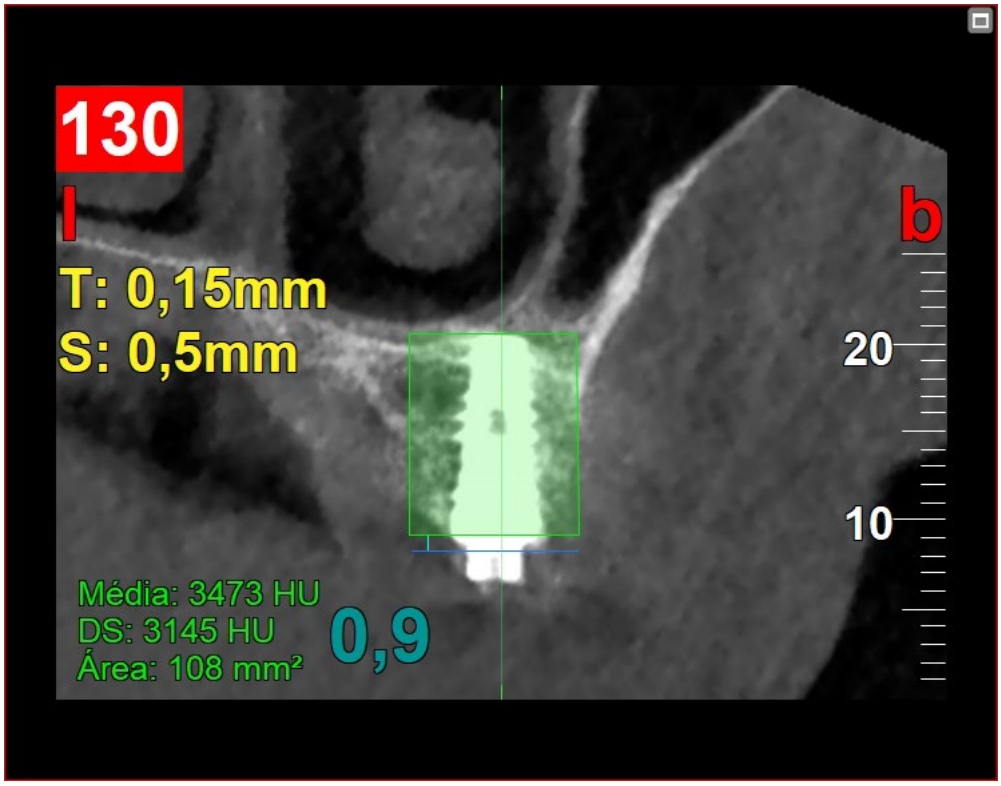


**Fig. 10 -** Four months post-operative follow-up CBCT considering bone height and density.

### 4.2.4 PILOT STUDY 1 RESULTS

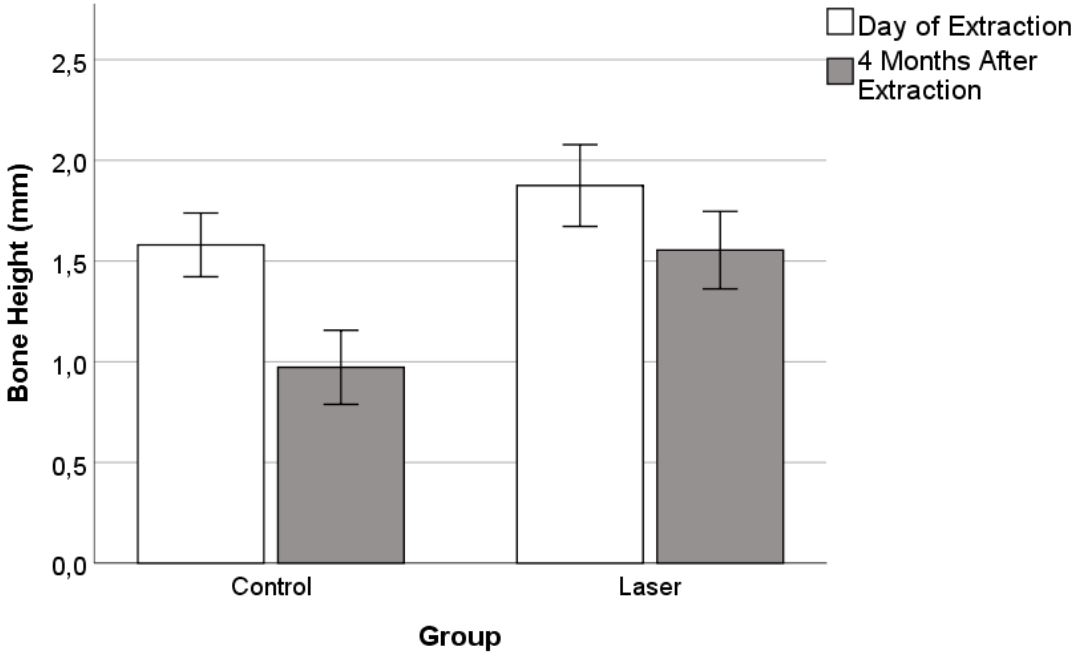


**Fig. 11 -** Variation in the mean and the error of the mean (error bars) of bone height (mm) for the several extraction sites (both represented, control and experimental groups) at two different time points: day of extraction (white bars) and 4 months after extraction (gray bars).

Although a reduction in the mean bone height (mm) has been observed 4 months after extraction for the elements of the control and the experimental groups, this reduction is two times less pronounced in the case of the experimental group (-0.321 mm) when compared with the control group (-0.608 mm), which seems to indicate the beneficial effect of the laser treatment when compared with the standard procedure Fig. 11.

| Control Group vs Experimental Group | Mean of the Differences | SD of the Differences | Test Statistics (*t*) | *p-value* |
|---|---|---|---|---|
| **Day of Extraction** | -0.295 | 0.256 | -1.151 | 0.255 |
| **4 Months After Extraction** | -0.582 | 0.266 | -2.188 | 0.034* |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | | | |

**Table 4 -** Comparison of control and experimental groups regarding the mean and standard deviation (SD) of bone height (mm) at the day of extraction and 4 months after extraction.

This observation was also confirmed by direct comparison between the control and experimental groups at the two different time points studied (Table III). On the day of extraction, no statistically significant differences were detected between the control and the experimental groups ($t$ = -1.151, $p$ = 0.255) in regards to bone height (mm), whereas 4 months after extraction, statistically significant differences were found between the control and the experimental groups ($t$ = -1.151, $p$ = 0.034) in regards to bone height (mm).

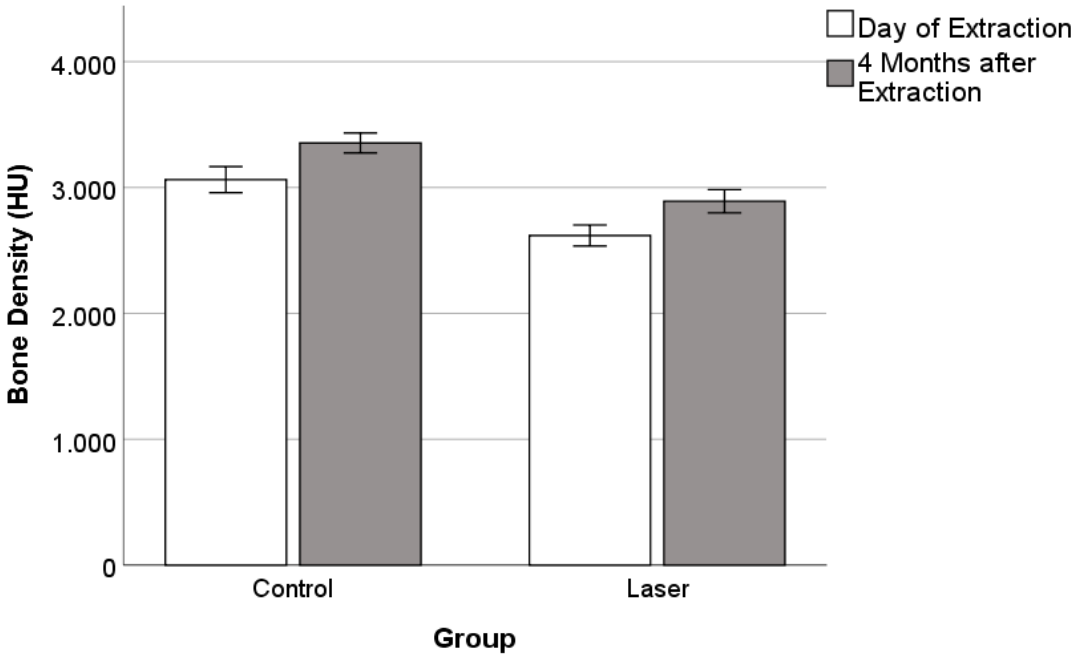


**Fig. 12 -** Variation in the mean and the error of the mean (error bars) of bone density (HU) for the several extraction sites (both represented, control and experimental groups) at two different time points: day of extraction (white bars) and 4 months after extraction (gray bars).

An increase in bone density (HU) was observed 4 months after extraction for the elements of the control and the experimental groups, which shows the positive effect of both the standard and laser procedures in the patient recovery.

| Control Group vs Experimental Group | Mean of the Differences | SD of the Differences | Test Statistics (*t*) | *p-value* |
|---|---|---|---|---|
| **Day of Extraction** | 443.900 | 133.426 | 3.327 | 0.002** |
| **4 Months After Extraction** | 462.527 | 121.412 | 3.810 | 0.000*** |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | | | |

**Table 5 -** Comparison of control and experimental groups regarding the mean and standard deviation (SD) of bone density (mm) at the day of extraction and 4 months after extraction.

Inferential statistics have revealed the presence of statistically significant differences between the control and experimental groups at the two different time points studied (Table IV). Although the difference in bone density (HU) between the control and experimental groups on the day of extraction was unexpected, the trend of increased bone density (HU) 4 months after extraction has been observed for both groups. Probably, a larger sample and a longer follow-up period would be needed to better detect any differences in bone density (HU) in this research setup.

## 4.3 OUTCOME 2 - INSERTION TORQUE

### 4.3.1 IMPLANT STABILITY

Dental implants require proper osseointegration for lasting aesthetic and functional rehabilitation. Stability is one of the requirements to achieving adequate osseointegration in implants, and is divided into two phases (Gahona *et al*., 2018). The first, primary stability, is clearly mechanical and consists in the strength and stiffness of the bone implant bonding by pressure at the time of insertion, determining whether or not it is subject to load. This stability reduces with time, as remodeling of the surrounding bone occurs. Subsequently, the second phase – also called biological – occurs, when new bone formation in direct contact with the implant surface forms (Cehreli *et al*., 2009; Gahona *et al*., 2018).

Primary stability of implants is commonly considered as a key factor for achieving successful osteointegration (Kim *et al*., 2021). Primary stability is influenced by various factors, such as the length and diameter of the implant, its design, the micro-morphology of the implant surface, the insertion technique and the congruity between the implant and the surrounding bone (Lozano-Carrascal *et al*., 2016; Norton, 2011). Micromovements greater than 50–150μm have a detrimental effect on bone formation around the implant surface, leading to the formation of fibrous tissue and, consequently, implant failure (Szmukler-Moncler *et al*., 1998).

A high insertion torque value implies sufficient primary stability of implants while a low value indicates low primary stability with greater possibility of early failure. As such, insertion torque measurement can be utilized in estimating the period with an optimal healed state suitable for a further load (Kim *et al*., 2021; Lozano-Carrascal *et al*., 2016).

Theoretically, it has been suggested that the bone is an elastic material before its yielding point, which is an indication that a certain level of strain can be tolerated, due to a relaxation effect (Campos *et al*., 2015; Szmukler-Moncler *et al*., 1998). On the other hand, once the strain in the bone exceeds the yielding point, numerous micro-fractures along with blood capillary overcom-pression provoke ischemic necrosis or in the worst case scenario, complete bone fracture (Bashutski *et al*., 2009). It has been acknowledged that ischemia and/or pressure necrosis have an impact on rapid bone resorption; however, reports suggest that the living bone can tolerate certain levels of overcompression (beyond the yield strain) without provoking negative bone responses (Bashutski *et al*., 2009; Campos *et al*., 2015).

### 4.3.2 SURGIC PRO2

Total insertion torque (N/cm) reflects the consumed electric current during tapping or implant insertion by a motor unit-connected computer and indirectly holds a given value in primary implant stability. The total insertion torque value is the sum of the true cutting resistance of bone during tapping or implant insertion, the friction and the contribution of bone shiver packing in deeper preparations.

The Surgic Pro2 micro-motor, produced by NSK (Japan), has significant size and weight reductions. Operability during treatment has been improved by moving the center of gravity closer to the head of the handpiece. This increases efficiency and alleviates the stress during prolonged operation, for strain-free, effortless operation.

Use of high-resolution color LED allows blood and soft tissue to be seen as if naturally-lit, thereby providing increased visibility during treatment. The irrigation pump provides consistent and steady flow, operating quietly in the background.

The insertion torque is aimed to fall within 30–50 Ncm and may be adjusted by using a larger implant or rotating the implant in the opposite direction if the value is out of the range (Baldi *et al*., 2018).


**Fig. 13 -** Positive effects of host-microbiome symbiosis (Kilian et al., 2016).

### 4.3.3 PILOT STUDY 2 PROTOCOL

Fourteen patients attending the combined oral surgery/implantology clinic at the Clitrofa – Centro Médico, Dentário e Cirúrgico, in Trofa - Portugal, scheduled for tooth extraction and immediate implant placement at the same surgical time, were tested according to the following protocol:

The maximum insertion torque value of each implant was recorded with Surgic Pro2 on the day of extraction and immediate implant placement.

The insertion torque automatically increased in single units increments until the operator was unable to rotate the implant due to friction, sometimes even before complete insertion of the implant.

### 4.3.4 PILOT STUDY 2 RESULTS

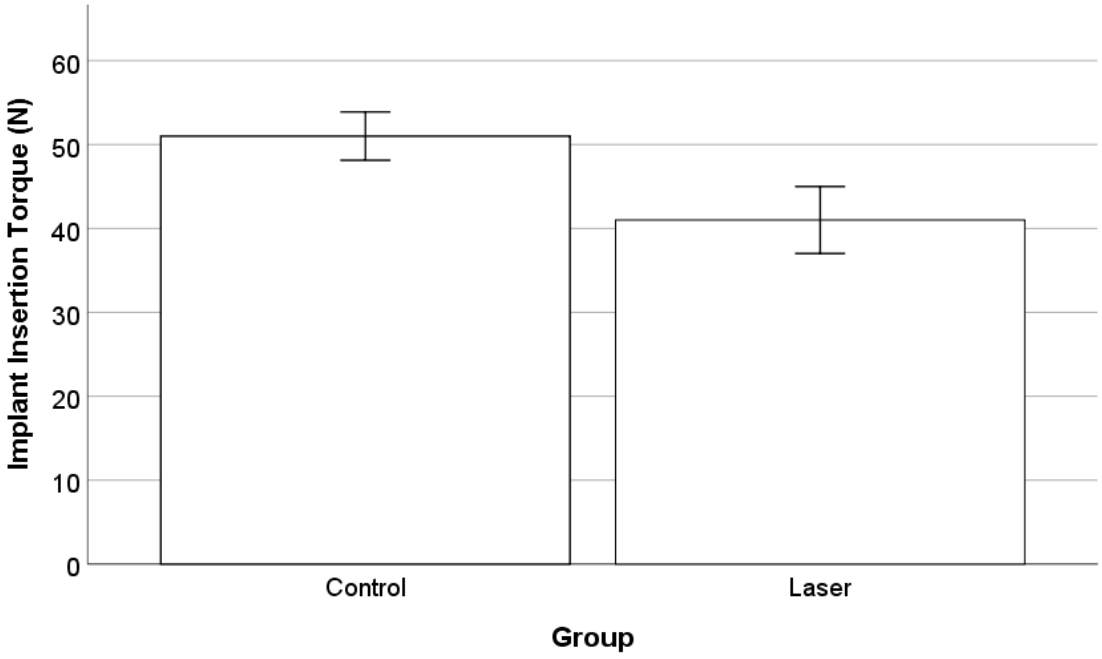


**Fig. 14 -** Variation in the mean and the error of the mean (error bars) of implant insertion torque (N) for the implants placed in the elements of the control and experimental groups.

Results presented in Fig. 14 show an apparent difference between the control and experimental groups regarding the mean implant insertion torque, with the laser presenting a lower implant insertion torque (41.00 ± 19.95 N), when compared with the control group (51.00 ± 14.36 N).

| Control Group vs Experimental Group | Mean of the Differences | SD of the Differences | Test Statistics (*t*) | *p-value* |
|---|---|---|---|---|
| **Implant Insertion Torque (N)** | 10.000 | 4.916 | 2.034 | 0.047* |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | | | |

**Table 6 -** Comparison of control and experimental groups regarding the mean and standard deviation (SD) of implant insertion torque (N) during implant placement.

The differences observed, which are statistically significant (Table V), are probably due to the previous differences detected in bone density (HU): the denser the bone, the higher implant insertion torque will be needed to place the implant in the correct position in the patient.

## 4.4 OUTCOME 3 - RESONANCE FREQUENCY ANALYSIS (RFA)

### 4.4.1 ASSESEMENT METHODS

Historically, the gold standard method to evaluate osseointegration was histologic analysis. Later, implant stability and osseointegration were clinically determined by tactile perception, radiographs, percussion test, reverse torque test, cutting torque resistance analysis, periotest, and RFA (Atsumi *et al*., 2007; Gahona *et al*., 2018).

Histologic analysis is not widely used though it is clinically accepted, due to unnecessary biopsies required for implant stability assessment. Radiographic analysis is a noninvasive method that can be performed at any stage of healing, yet changes in radiographic bone level cannot precisely indicate implant stability. Percussion tests provide a ringing sound as a sign of good osseointegration and are not reliable as they provide poor qualitative information (Atsumi *et al*., 2007).

Cutting torque resistance analysis utilizes energy that correlates to bone density further determining implant stability. It cannot assess secondary stability and is not frequently used as a diagnostic aid, as the lower limit value that denotes potential failure of implant has not been established (Atsumi *et al*., 2007; Sennerby and Meredith, 2008).

Reverse torque test gives information on degree of bone to implant contact of any given implant and is not widely used as it can provide information as to all or no outcome (osseointegrated or failed) and it cannot quantify the degree of osseointegration (Atsumi *et al*., 2007; Sennerby and Meredith, 2008).

Dental periotest has been thoroughly studied and advocated as a reliable method to determine implant stability. Readings of -8 to + 50 are interpreted. Successfully integrated implants have yielded a wide range of periotest values. These variations suggest that for implants there is no absolute value that is considered acceptable. Periotest cannot diagnose a borderline case or an implant in the process of osseointegration (Atsumi *et al*., 2007; Sennerby and Meredith, 2008).

The RFA method was first described in 1996 for implant stability measurement. This technique measures the resonance of a transducer that is attached to implants to correlate with micromobility or displacement, which in turn is determined by the bone density. The RFA technique provides clinically relevant information about the state of the implant–bone interface at any stage after implant placement. It can be used as an

additional parameter to support decision-making during implant treatment and follow-up (Sennerby and Meredith, 2008).

Correlation between implant stability quotient (ISQ) and bone-implant contact is assessed utilizing an RFA device, stating that a significant positive correlation exists between the RFA and bone-implant contact (BIC) values and more bone contact with implant surface implies higher implant stability (Scarano *et al*., 2006).

The clinical range of ISQ is normally 55-80, higher values are generally observed in the mandible than in the maxilla. The ISQ scale has a non-linear correlation to micro mobility; high stability means >70 ISQ, between 60-69 corresponds to medium stability and < 60 ISQ is considered as low stability. If the initial ISQ value is high, a small drop in stability normally levels out with time. A big drop in stability or decrease should be taken as a warning sign. Lower values are expected to be higher after the healing period. The opposite could be a sign of an unsuccessful implant and action should be considered.

### 4.4.2 OSSEO 100

The Osseo 100 produced by NSK (Japan) is a non-invasive, objective, accurate and repeatable device. The MulTipeg™ is attached to the implant and it screws effortlessly into the implant's internal threads (approximately 6-8 N/cm of torque). The peg responds by magnetic pulses and vibrates due to the stiffness in the contact area between the bone and the implant surface. An ISQ value is generated and shown on the display; with units ranging from 1 to 100, with higher values of the ISQ indicating higher implant stability (Kim *et al*., 2021).


**Fig. 15 -** Positive effects of host-microbiome symbiosis (Kilian *et al*., 2016).

### 4.4.3 PILOT STUDY 3 PROTOCOL

Fourteen patients attending the combined oral surgery/implantology clinic at the Clitrofa – Centro Médico, Dentário e Cirúrgico, in Trofa - Portugal, scheduled for tooth extraction and immediate implant placement at the same surgical time, were tested according to the following protocol:

Measurements will be performed at three different times, namely immediately after implant placement, 2 months after surgery and 4 months after surgery. The ISQ value was measured twice in each of the three measurements and the mean considered (Sennerby and Meredith, 2008).

### 4.4.4 PILOT STUDY 3 RESULTS

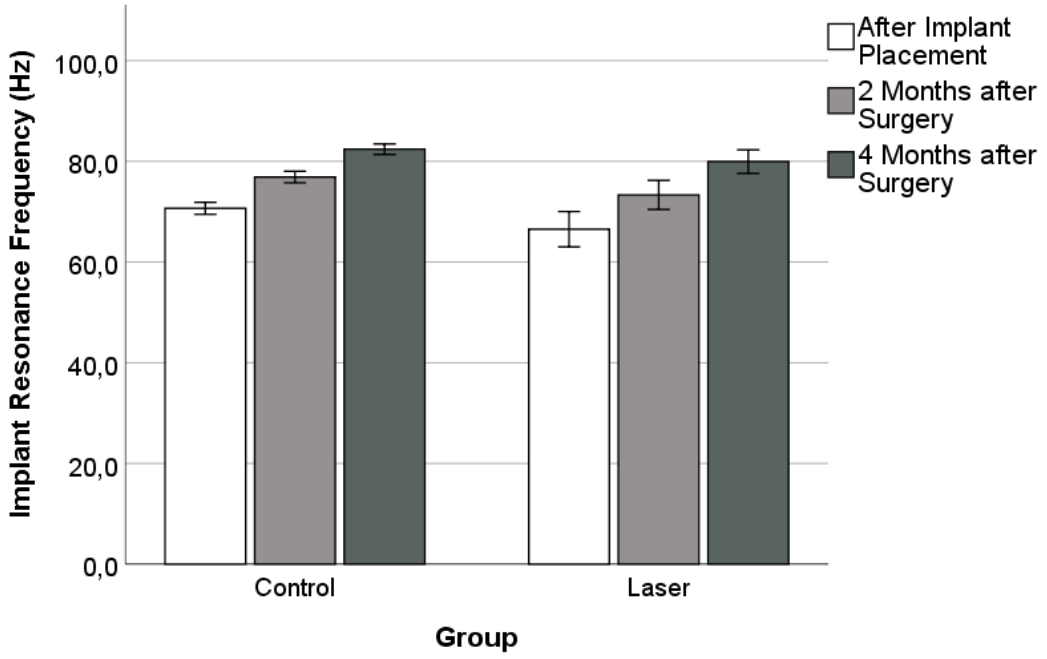


**Table 7 -** Variation in the mean and the error of the mean (error bars) of the implant resonance frequency (Hz) for the implants placed in the elements of the control and experimental groups, at three different time points: immediately after implant placement (white bars), 2 months after surgery (gray bars) and 4 months after surgery (dark gray bars).

An increase in implant resonance frequency (Hz) has been observed throughout the three time points analyzed, both in the control and in the experimental groups, showing the progressive osteointegration of the implant in the implant placement site.

| Control Group vs Experimental Group | Mean of the Differences | SD of the Differences | Test Statistics (*t*) | *p-value* |
|---|---|---|---|---|
| **After Implant Placement** | 4.140 | 3.698 | 1.119 | 0.269 |
| **2 Months After Surgery** | 3.560 | 3.111 | 1.144 | 0.258 |
| **4 Months After Surgery** | 2.460 | 2.561 | 0.961 | 0.342 |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | | | |

**Table 8 -** Comparison of control and experimental groups regarding the mean and standard deviation (SD) of implant resonance frequency (Hz) immediately after implant placement, 2 months after surgery and 4 months after surgery.

Although the differences between the control and experimental groups regarding the implant resonance frequency (Hz) have not been considered statistically significant in any of the time points studied, as can be observed in Table VI, it is interesting to notice that the magnitude of the differences has shown to decrease with the increase in time. This tendency suggests that the rate of osteointegration, as assessed by implant resonance frequency technique, is higher in the laser treatment, when compared with the control treatment.

## 4.5 OUTCOME 4 - CLINICAL SIDE EFFECTS

### 4.5.1 RISK FACTORS

Following the introduction of immediate implant placement protocols, both patients and clinicians have demonstrated increased interest in this technique. Immediate implant placement is a method that decreases the number of surgeries and therefore the total treatment time, minimizes bone resorption following a tooth extraction and thus maintains the periodontal architecture leading to better esthetic treatment outcomes, and achieves optimal implant orientation and positioning (Chatzopoulos and Wolff, 2022; Chrcanovic *et al*., 2014; Lemes *et al*., 2015). In addition, this treatment approach results in higher patient satisfaction than the conventional/delayed placement protocol (Chatzopoulos and Wolff, 2022; Chrcanovic *et al*., 2014; Lemes *et al*., 2015).

On the other hand, immediate implants have also been associated with increased surgical complications, poor esthetic outcomes due to gingival recessions and conflicting findings regarding their failure rates (Grandi *et al*., 2013). A dental implant is considered failed when it demonstrates clear signs or symptoms that it requires removal (Chrcanovic *et al*., 2016).

Early implant failure is associated with poor osseointegration and the inability to achieve optimum bone to implant contact, while late implant failure is primarily a result of biological complications which are characterized by the inability to maintain osseointegration (Chrcanovic *et al*., 2016).

The risk factors can be subdivided into iatrogenic, material-associated, and patient-related factors (Staedt *et al*., 2020). Side effects during surgery include heat-induced necrosis, poor primary stability, and incorrect positioning (Staedt *et al*., 2020). The implant’s geometry, including the implant’s dimensions and its macro-design, as well

as the type of prosthetic treatment does affect loading distribution and in consequence the dental implant's survival rate (de Souza *et al*., 2013; Staedt *et al*., 2020). Local risk factors include significant plaque accumulation, gingivitis, tight implant-tooth contact, bone quality and quantity, poor oral hygiene, periodontal disorders, and chronic occlusal trauma. Also, systemic factors like xerostomia, osteoporosis, cardiovascular diseases, and diabetes mellitus are reported to influence the patients' wound-healing capability (de Souza *et al*., 2013; Staedt *et al*., 2020).

The concept of immediate implant loading has recently become popular due to less trauma, reduction in overall treatment time, decrease in hard and soft tissue resorption, increase in patient's acceptance, along with better function, aesthetics and it also brings a psychological benefit (Singh *et al*., 2012).

The initial stability of the implant is essential for early/ immediate loading. The minimum insertion screw has to be equal or superior to 32 N/cm and the micro movement of the implant should not exceed 150 *um*. Bruxism and the lack of primary stability of the implants are contraindications for the immediate loading (Singh *et al*., 2012).

The major key factor for the success of implant therapy is appropriate patient selection. Therefore, it is crucial for the dental practitioner to recognize the risks of implant failure, identify patients and sites that are suitable for dental implants and define a treatment plan accordingly to ensure the long-term clinical success of an implant placement. In addition, immediate implant placement into extraction sockets has become a widely acceptable treatment option (Chatzopoulos and Wolff, 2022).

### 4.5.2 PILOT STUDY 4 PROTOCOL

Fourteen patients attending the combined oral surgery/implantology clinic at the Clitrofa – Centro Médico, Dentário e Cirúrgico, in Trofa - Portugal, scheduled for tooth extraction and immediate implant placement at the same surgical time, were tested according to the following protocol:

Potential side effects: Pain rating will be measured on a scale from 0 to 10 (0-no pain to 10-unspeakable pain). Measurements will be performed at three different times, namely during treatment, and on days 8 and 30 after surgery. Other complications such as bleeding, swelling, trismus, implant failure and bone loss will also be monitored.

### 4.5.3 PILOT STUDY 4 RESULTS

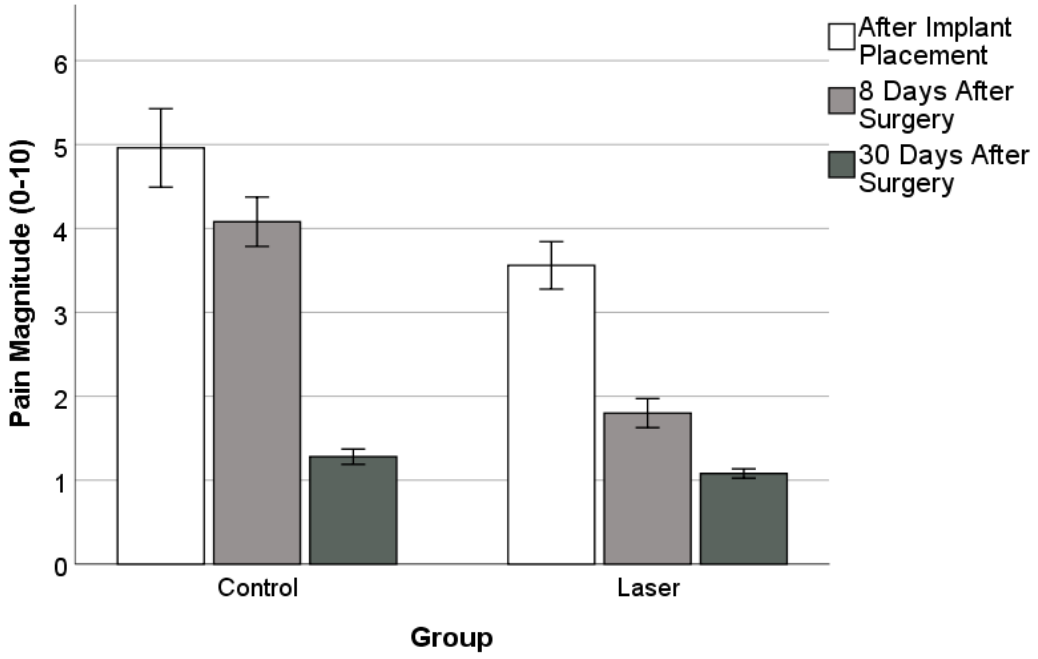


**Fig. 16 -** Variation in the mean and the error of the mean (error bars) of the pain magnitude (0-10 international pain scale) for the implants placed in the elements of the control and experimental groups, at three different time points: immediately after implant placement (white bars), 8 days after surgery (gray bars) and 30 days after surgery (dark gray bars).

| Control Group vs Experimental Group | Mean of the Differences | SD of the Differences | Test Statistics (*t*) | *p-value* |
|---|---|---|---|---|
| **After Implant Placement** | 1.400 | 0.546 | 2.562 | 0.014* |
| **8 Days After Surgery** | 2.280 | 0.341 | 6.683 | 0.000*** |
| **30 Days After Surgery** | 0.200 | 0.068 | 1.868 | 0.068 |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | | | |

**Table 9 -** Comparison of control and experimental groups regarding the mean and standard deviation (SD) of pain magnitude (0-10 international pain scale) immediately after implant placement, 8 days after surgery and 30 days after surgery.

The assessment of patient pain magnitude (0-10 international pain scale) following the two different implant placement procedures, has revealed that laser treatment produces statistically significant lower pain than the standard procedure, namely in the times immediately after implant placement and 8 days post surgery (Table 9, Fig. 16). This observation shows the less invasive profile of laser treatment and its higher general acceptance by the patients.

| | Control Group | Experimental (Laser) Group | Total |
|---|---|---|---|
| **Absence (Frequencies)** | No: 17 (68.0%)<br>Yes: 8 (32.0%) | No: 12 (48.0%)<br>Yes: 13 (52.0%) | No: 29 (58.0%)<br>Yes: 21 (42.0%) |
| **Bleeding (Frequencies)** | No: 25 (100.0%)<br>Yes: 0 (0.0%) | No: 14 (56.0%)<br>Yes: 11 (44.0%) | No: 39 (78.0%)<br>Yes: 11 (22.0%) |
| **Swelling (Frequencies)** | No: 8 (32.0%)<br>Yes: 17 (68.0%) | No: 25 (100.0%)<br>Yes: 0 (0.0%) | No: 33 (66.0%)<br>Yes: 17 (34.0%) |
| **Trismus (Frequencies)** | No: 25 (100.0%)<br>Yes: 0 (0.0%) | No: 25 (100.0%)<br>Yes: 0 (0.0%) | No: 25 (100.0%)<br>Yes: 0 (0.0%) |
| **Implant Failure (Frequencies)** | No: 18 (72.0%)<br>Yes: 7 (28.0%) | No: 24 (96.0%)<br>Yes: 1 (4.0%) | No: 42 (84.0%)<br>Yes: 8 (16.0%) |
| **Bone Loss (Frequencies)** | No: 19 (76.0%)<br>Yes: 6 (24.0%) | No: 25 (100.0%)<br>Yes: 0 (0.0%) | No: 44 (88.0%)<br>Yes: 6 (12.0%) |

**Table 10 -** Complications associated with implants placed during and after implant placement.

| Control Group vs Experimental Group | Test Statistics (*U*) | *p-value* |
|---|---|---|
| **Absence of Complications** | 250.000 | 0.156 |
| **Bleeding** | 175.000 | 0.000*** |
| **Swelling** | 100.000 | 0.000*** |
| **Trismus** | 312.500 | 1.000 |
| **Implant Failure** | 237.500 | 0.022* |
| **Bone Loss** | 237.500 | 0.010** |
| ** moderately significant to 0.05 level; ** significant to 0.01 level; *** highly significant to 0.001 level.* | | |

**Table 11 -** Complications associated with implants placed during and after implant placement.

The assessment of complications following the two different implant placement procedures, has revealed that laser treatment produces statistically significant lower swelling, implant failure and bone loss than the standard procedure, whereas it produces higher bleeding than the latter (Tables VIII and XIX). Altogether, these results indicate that patient complications following implant placement are less prevalent in laser treatment, than in the standard procedure.

# CHAPTER 5 – DISCUSSION

A challenging dilemma in implant dentistry is when to place a dental implant immediately following an extraction or to opt for a delayed placement once the soft and hard tissues have healed. The literature remains controversial. Implant treatment protocols have shown both failures and complications (Chatzopoulos and Wolff, 2022).

Various studies have examined the effect of systemic medical conditions on the survival rates of dental implants. Although osteoporosis, human immunodeficiency virus, cardiovascular disease, hypothyroidism, bleeding disorders and diabetes have been identified as conditions that may affect implant survival, the available literature is inconclusive (Bornstein *et al*., 2009).

Some factors have been investigated in the literature for their role in implant survival including age, gender, implant length and diameter, implant location, patient's medical condition, smoking habits, implant location as well as bone quality (Barbosa *et al*., 2020).

In a large retrospective study of 30959 implants, Lin and colleagues (2018) demonstrated that males, patients aged ≥41 years, and mandibular anterior location were risk factors for early implant loss, whereas males, patients aged ≥41 years, bone augmentation, and short implants were risk factors for late implant loss (Lin *et al*., 2018). Smoking has a detrimental effect on implant survival that is mainly attributed to the lower bone formation rate and longer mineralization time as well as the abnormal angiogenesis, that leads to decreased vascularization and remodeling (Lin *et al*., 2018). A recent systematic review and meta-analysis showed that implants in smokers exhibited a 140.2% higher failure risk when compared to non-smokers, as well as an increased marginal bone loss (Mustapha *et al*., 2021).

A different parameter that has been associated with a possible higher implant failure in immediately placed dental implants is the presence of apical periodontitis. Early studies reported that immediate implants are contraindicated in the case of periapical and periodontal lesions, due to the risk of microbial interference (Chrcanovic *et al*., 2014). Recent evidence suggests that immediate implants in sites with periapical and periodontal pathology result in similar clinical outcomes compared to those placed in healthy sites, providing that meticulous cleaning, socket curettage/debridement, and

chlorhexidine 0.12% rinse are performed prior to implant placement (Chatzopoulos and Wolff, 2022).

Evaluation of bone density was performed initially by subjective analysis; later studies correlated HU and objective assessment of bone density (Silva *et al*., 2012). There is a strong relationship between high bone density and a high rate of success with implants. There is also good correlation between high bone density and the primary stability of the implants (Silva *et al*., 2012).

The mean bone density value of the posterior maxillary region reported by Fuster-Torres *et al*., (2011) was 464 HU for 25 implant sites, while Norton and Gamble (2001) reported the mean bone density in 27 maxillary implant sites to be 417 HU. Turkyilmaz and McGlumphy (2008) and Shapurian *et al*., (2006) performed similar studies, and their results were 403 HU for 70 implant sites and 333 HU for 54 implant sites, respectively.

In addition, Turkyilmaz and McGlumphy (2008) and Isoda *et al*., (2012) reported statistically significant correlations between HU and the parameters of primary stability, IT and RFA.

The implant stability is a critical factor to consider in the prosthetic rehabilitation, since all loading protocols require a stable mechanical connection between the implant and the bone; between insertion torque and RFA when evaluating the stability of implants positioned in the maxilla and mandible and in bones of different densities (Gahona *et al*., 2018). The minimum insertion torque values for unitary implants and multiple splinted implants are 30 and 20 Ncm, respectively, for immediate loading (Kim *et al*., 2021).

Therefore, it may be possible to overcome the risk of poor stability in areas of low-density bone through procedural techniques, such as using an implant with a larger diameter. It can be interpreted that the factor that affects primary stability during the implant procedure is more influenced by bone quality than implant type (Kim *et al*., 2021).

Determining factors of primary stability are macro-design features and micromorphology of the implant, the insertion technique, and proximity between the implant and the surrounding bone (Kim *et al*., 2021). However, previous studies have shown conflicting opinions regarding the influence of implant geometry on primary stability. While some authors concluded that the length and diameter of the implants do not significantly influence ISQ values (Bilhan *et al*., 2010), others demonstrated a positive correction between implant length or diameter and ISQ, particularly where poor bone quality was detected (Barikani *et al*., 2013).

Early DIF is associated with impaired bone healing and a reduced amount of implant primary stability by insufficient bone-to-implant contact (Staedt *et al*., 2020). Factors like heat-induced necrosis and incorrect positioning may lead to impaired osseointegration resulting in early implant loss. Late DIF is defined by a reduction of implant stability after a latency of 6 months, this is thought to be a multifactorial process, as both implant and patient-related factors influence the implant's long-term survival. On the one hand, loading distribution is affected by the implant's geometry as well as the type of prosthetic treatment in particular different occlusion concepts. On the other hand, local risk factors like plaque accumulation, gingivitis, bone quality and quantity, oral hygiene, periodontal disorders, and chronic occlusal trauma determine the implant's long-term outcome (Staedt *et al*., 2020).

Implants are considered successful if there is less than 0.2 mm bone loss annually after the first year of loading, if they are clinically immobile, if there is no peri-implant radiolucency and if there is no persistent and/or irreversible pain, infection, neuropathies or paresthesia (Chatzopoulos and Wolff, 2022).

The results of this study show that a comprehensive laser post-extraction with immediate implant placement procedure consisting of degranulation, disinfection, decortication, de-epithelialization, clot stabilization and photobiomodulation using Er:YAG and Nd:YAG wavelengths significantly improves bone healing and prevents bone loss after implant placement at 4-month follow-ups, when compared to a standard post-extraction procedure.

The Nd:YAG PBM laser deep penetration is most likely the cause for the observed differences, by promoting osteoblast differentiation, as demonstrated by the higher expression of osteocalcin in experiments in rats (Mergoni *et al*., 2016). Studies comparing the outcome of guided tissue regeneration, alone or in combination with Nd:YAG PBM, for treatment of furcation defects or periodontal defects ,showed significantly more improvement in pocked depth, clinical attachment level, horizontal probing depth, and alkaline phosphatase levels in lased than in non-lased group (Križaj *et al*., 2021).

Deana *et al*., (2018) concluded that osteoblasts are susceptible to PBM, but most of the light parameters employed by different authors unfortunately had little to no influence on proliferation and very high doses had dangerous effects on cell homeostasis, while Escudero *et al*., (2019) pointed out that many data supports the positive effects of PBM on bone regeneration, by accelerating this process. More

generally, Dompe *et al*., (2020) suggested that PBM can induce cell proliferation, enhance stem cell differentiation, and improve healing and tissue repair processes.

The statistically higher implant insertion torque of the standard post-extraction procedure when compared to the laser post-extraction procedure, has been attributed to the differential positioning of the implants between the control and experimental groups, which may be prevented by the use of a larger sample and more homogeneous control and experimental groups with respect to patient sociodemographic characteristics and tooth position.

Finally, the laser post-extraction procedure has revealed statistically significant lower prevalence of swelling than the standard post-extraction procedure (0% *versus* 68%), implant failure (4% *versus* 28%) and overall complications registered (48% *versus* 68%). Regarding the patients' self-perception of pain, the laser post-extraction procedure is associated with statistically significant lower pain when compared to the standard post-extraction procedure, namely in the evaluations made after implant placement and 8 days after surgery. These clinical findings show the low-invasiveness and low-health risk profile of laser post-extraction procedure when compared to the standard post-extraction procedure.

Altogether, the data collected suggests a superior performance of laser post-extraction procedure against the standard post-extraction procedure, and reinforces its potential for application in the clinical environment.

# CHAPTER 6 – CONCLUSION

A comprehensive laser post-extraction procedure with immediate implant placement protocol using Er:YAG and Nd:YAG laser wavelengths for degranulation, disinfection, decortication, de-epithelialization, clot stabilization and photobiomodulation was developed and compared to a standard post-extraction procedure through a randomized controlled trial, where the following comparison parameters were considered: bone loss (mm), bone density (HU), implant insertion torque (N), implant resonance frequency (Hz) and clinical side effects (patients' self-perception of pain, clinical complications during follow-up).

The laser post-extraction procedure has shown statistically lower bone loss than the standard post-extraction procedure 4 months after implant placement (-0.321 mm versus -0.608 mm, respectively) while comparable increases on bone density (HU) and implant resonance frequency (Hz) to the standard post-extraction procedure. The statistically higher implant insertion torque of the standard post-extraction procedure when compared to the laser post-extraction procedure, has been attributed to the differential positioning of the implants between the control and experimental groups, and therefore does not pose any clinical significance.

Finally, the laser post-extraction procedure has revealed statistically significant lower prevalence of swelling than the standard post-extraction procedure (0% *versus* 68%), implant failure (4% *versus* 28%) and overall complications registered (48% *versus* 68%). Regarding the patient's self-perception of pain, the laser post-extraction procedure is associated with statistically significant lower pain when compared to the standard post-extraction procedure, namely in the evaluations made after implant placement and 8 days after surgery.

Altogether, the data collected suggests a superior performance of laser post-extraction procedure against the standard post-extraction procedure, and reinforces its potential for application in the clinical environment. Future studies comprising a higher number of implants and complementary assessment techniques will be attempted.

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