COMPLUTENSE UNIVERSITY OF MADRID

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EFFECT OF MELATONIN IN THE TREATMENT OF PERI-IMPLANT DISEASES

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1. MAIN DATA

• **Title:** EFFECT OF MELATONIN IN THE TREATMENT OF PERI-IMPLANT DISEASES

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2. BACKGROUND

Peri-implant diseases are defined as the host response to the formation of a biofilm on the implant, which initially includes various inflammatory reactions in the soft tissue surrounding the implant, and which can subsequently progress and cause loss of bone support; that is, it includes inflammatory pathological changes in the tissues surrounding an implant subjected to load.
[1].

Two types of peri-implant diseases can be considered: [2]:

“Peri-implant mucositis” was the term proposed for the reversible inflammation of the soft tissues surrounding functioning implants (Albrektsson and Isidor, The First European Workshop on Periodontology, 1994)3 .

“Peri-implantitis” was introduced in the late 1980s and was later defined as[4] an inflammatory process affecting the soft and hard tissues surrounding a functioning osseointegrated implant, resulting in loss of supporting bone.
[3].

Peri-implant mucositis and peri-implantitis have become an increasing problem in recent years, due to the increased and more frequent use of endosseous dental implants as part of prosthetic rehabilitation. According to the Sixth European Consensus on[5] Periodontology (2008), its incidence is between 28-56%.

The etiology of this disease is not clear, although the increased presence of gram-negative microorganisms and spirochetes has been determined when associated with pathogenic bacteria. In other cases, bone loss can be caused by occlusal trauma or peri-implant overload. Genetic and systemic factors have also been described, as well as toxic habits that could influence the development and progression of this disease. Peri-implantitis is the main challenge facing dentists today. Bone loss occurs progressively and can cause early implant failure if appropriate treatment is not established.

It is known that the large increase in interleukins (IL-1, IL-6, IL-8 and IL-17) from cell types in the injured area triggers the production of the first symptoms derived from inflammation[6] , mostly caused by IL- 7,8
[6] . IL-6 plays an important role in leukocyte recruitment during the acute inflammation phase8,9 . IL-6 also stimulates bone resorption and appears to be involved in the early stages of osteoclastogenesis10 .

Melatonin can be defined as a hormone with paracrine, autocrine and antioxidant actions that exerts several actions dependent and independent of its receptors11,12 . Its first effects were described in 1917 by McCord and Allen, but it was not until 1958 when it was isolated and identified by Lerner, Case and Takahashi12 . This molecule is mainly synthesized in the pineal gland until 30 years of age. Melatonin production is genetically determined and varies between individuals, and can reach 200 pg/ml during the dark phase,

Its basal daytime level is about 10 pg/ml. It is also synthesized in other regions of the body such as the retina, the gastrointestinal tract, the skin, the bone marrow and lymphocytes13 .

One of the main physical-chemical characteristics of melatonin is its high diffusion capacity, since it is a very lipophilic molecule. This property allows melatonin to cross all morphophysiological barriers and penetrate all cells and cellular spaces13,14 . It also has a very high redox potential of 0.74 V, which determines an antioxidant activity far superior to that of other known antioxidants11,15,16 . There is general agreement that melatonin is a non-toxic molecule. It produces intense anti-apoptotic signals even in situations of ischemia and has cytoprotective properties that have been described for the treatment of neurodegenerative disorders. It also stimulates the immune system and has oncosuppressive properties that still today represent an extensive field of research13 .

Melatonin has already been used successfully after radical oral surgery interventions due to its antioxidant action and the stimulating and protective effects of intracellular enzymes involved in the healing process12,17 .
It has also been shown to regulate functions unrelated to the endocrine system and plays an important role in pain modulation. Several mechanisms have been proposed for its function, related to membrane receptors, nuclear receptors, and by scavenging free radicals in a receptor-independent manner15 .

Some studies in oral surgery support the usefulness of melatonin as an anti-inflammatory and protector against infections. Although its action would be amplified when the free radicals of NO (nitric oxide) resulting from tissue damage are already present in the injured area18 . In addition, the application of melatonin in the post-extraction alveolus favors tissue healing due to the properties of this molecule19,20 .

Other studies have shown that exogenous administration of melatonin above the body's basal levels stimulates the immune system21,22
increasing the proliferation of T21 lymphocytes , NK cells20,21 and granulocytes, and the antibody response26,27 . The activation of T lymphocytes increases the release of inflammatory cytokines such as interferon-ÿ, TNF-ÿ, IL-1, IL-1ÿ, IL-2, IL-6 and IL- 1228,29,30,31,32,33,34. In addition, it has been proposed that it induces the production of NO35 .

This stimulation of the immune and inflammatory system dependent on the concentration of melatonin35,36 would be responsible for the inflammatory response23 and the defense of the body against bacterial and viral infections35,38 .

The result of these studies shows that, in general, melatonin could act by stimulating the immune system in basal conditions or states of immunosuppression, for a more effective early immune response. However, in the presence of an exacerbated immune or inflammatory response, melatonin could exert a negative regulation by acting as an anti-inflammatory. Therefore, melatonin could be considered a pleiotropic molecule with varied and complex effects on the immune system.

immune and inflammatory system. These immunomodulatory and anti-inflammatory actions of melatonin have been successfully studied in several experimental models, and there is general agreement that melatonin can be administered with insignificant side effects39 .

On the other hand, melatonin seems to contribute to the neoformation of bone since it stimulates the differentiation of preosteoblasts, which are transported from the bone marrow to the alveolar bed through the vascular system. In addition, it stimulates the gene expression of certain proteins of the bone matrix and stimulates the activity of osteoblasts40,41 .

There are studies applied to osseointegrated implants that have observed an earlier differentiation of osteoblasts caused by melatonin, which accelerates the synthesis and mineralization of the osteoid matrix after 2 weeks27 and 4 weeks40, as well as a significant increase in the width and length of cortical bone . 42,43 This rapid increase in bone formation indicates that melatonin acts during bone remodeling at two levels simultaneously. 42,43,44 In histological analysis, the local application of 3 mg of melatonin has been shown to increase trabecular formation by directly stimulating osteoblasts from the endosteum . 45,46

3. JUSTIFICATION OF THE STUDY

Peri-implant diseases are defined as those inflammatory reactions
pathological changes in the soft tissue surrounding the loaded implant, which may subsequently progress and lead to loss of bone support.

The diagnosis of this pathology should be confirmed by evaluating the clinical signs of mucosal inflammation, the presence of bleeding and/or suppuration on careful probing, increased probing depth compared to previous records, and bone loss of the crestal bone greater than the initial bone remodeling processes.

Peri-implant bone loss due to infection is the most common and complex problem in implantology. This bone loss, caused by the presence of pathogenic microorganisms, is associated with localized tissue inflammation.
irreversible. The problems resulting from this pathology make the prognosis of implants difficult, leading in many cases to their loss. Furthermore, there is currently no treatment for this disease. Its diagnosis is made by peri-implant probing and radiographic evaluation of the bone level.

Treatment protocols for peri-implantitis are not clearly established, even less so when the etiology of this disease is difficult to determine and in many cases could be multifactorial. As a standard, it has been proposed to eliminate all occlusal traumatic factors that could cause progression of bone loss due to overload. A basic periodontal treatment should then be carried out by disinfecting the surface of the implants and removing granulation tissue if present. In this way, the prostheses are temporarily removed for a variable period of between 1 and 2 weeks to avoid loading the implant.

implant and promote local hygiene and adequate healing of peri-implant tissue.

The etiology of the disease may determine that the process of bone loss continues to occur, although at a slower rate. The establishment of the disease as well as its progression may be due to systemic diseases and patient conditions, systemic medications, tissue healing processes, tissue renewal and tissue response to clinical interventions, trauma to orofacial structures, local diseases affecting teeth, periodontium, bone and mucosa, biomechanical factors, tissue morphology and phenotype and iatrogenic factors.

In some cases, bone substitutes and biomaterials have been proposed to try to recover bone loss in order to regenerate the bone defect that has occurred. However, predictable results have not been found in all situations and long-term results are still controversial.

Considering the beneficial results of melatonin obtained in the studies previous studies, investigated using different study models and through various evaluation methods, the present investigation of its antimicrobial, anti-inflammatory and osteogenic properties in patients is recommended.

There is sufficient scientific evidence about the usefulness and safety of melatonin as an antibacterial, anti-inflammatory and analgesic in situations of inflammatory pain and as an osteodifferentiator and early osteogenic.

This research will provide the quantification of antimicrobial and anti-inflammatory properties in a known model of inflammation caused by infection in patients with implants. It also aims to offer a therapeutic alternative in terms of bone formation in a situation that is currently difficult to predict, such as peri-implant bone defects.

The dissemination of the results of this research would allow us to offer the first effective, simple and economical therapeutic alternative with a high success rate that achieves peri-implant bone regeneration, a decrease in inflammation and pathogenic bacterial species. The single application of local melatonin in the same session as the peri-implant treatment would allow the reduction of pharmaceutical and biomaterial costs as well as the patient's recovery time. In this way, the antibacterial, anti-inflammatory and early osteogenic properties will allow patients with destructive bone pathology caused by bacteria to benefit.

4. HYPOTHESIS

There is an increased incidence of bone loss in osseointegrated implants.

The presence of Gram-negative pathogenic bacteria is the main etiological factor in peri-implant bone loss.

Regenerative treatment of bone defects is not predictable using conventional techniques and biomaterials.

Several studies have shown that melatonin has short-term anti-inflammatory and antimicrobial properties. In addition, due to its early osteogenic potential
could improve local bone tissue conditions, especially bone density, which is the most important factor for proper osseointegration.

Based on the above, we formulate the following main hypothesis:

Melatonin applied locally to peri-implant defects produces favorable differences in the treatment of peri-implant diseases in terms of the reduction of pathogenic bacterial species, decrease in mediators and signs of inflammation, and acceleration of local early osteogenesis with respect to conventional peri-implant treatment.

5. OBJECTIVES

Based on the previous hypothesis, this phase IV randomized pilot clinical study is proposed to be carried out.

The main objective of this research protocol is to evaluate the demonstrated effects of melatonin on the peri-implant sulcus of patients requiring treatment of peri-implant pathology with bone loss.

To this end, the following secondary objectives are established:

- To establish whether there are statistically significant differences in the presence of bacterial species after applying local melatonin compared to the control.
- To determine whether there are statistically significant differences in the levels of interleukin-1ÿ, 2, and 6 depending on the application of local melatonin versus the
control.
- To compare whether there are statistically significant differences in probing depth after peri-implant treatment depending on whether local melatonin or the control treatment is applied.
- To contrast whether there are statistically significant differences in the levels of peri-implant crestal bone after peri-implant regeneration depending on whether the treatment is performed with local melatonin or without it.
- Assess the appearance of complications associated with melatonin in the
postoperative.

6. MATERIAL

6.1. POPULATION AND SAMPLE

A. Population

The subjects who will participate in the study will be patients selected from the Department of Oral and Maxillofacial Medicine and Surgery of the Faculty of Dentistry of the Complutense University of Madrid. The cases will be those patients with an indication for peri-implant treatment, in good health (Category I and II of the American Society of Anesthesiologists [ASA]), of both sexes, aged between 40 and 85 years.

Inclusion criteria

- Patients of both sexes with implants with peri-implant disease according to the criteria of the Classification of Periodontal and Peri-implant Diseases and Conditions of the 2017 World Workshop.

- Ages between 40 and 85 years.

- Health status (ASA Category I and II).

- No history or metabolic or systemic diseases that affect the bone or the healing process of the surgical area, such as diabetes or osteogenesis imperfecta.

- Not suffer from autoimmune diseases.

- Not suffer from coagulation or healing disorders.

- Implants of the same diameter, distributed bilaterally.

- Patients with one or more fully erupted teeth.

- Meet the inclusion criteria and agree to sign the consent informed.

Exclusion criteria

- Tobacco use.

- Use of contraceptive or hormonal medications.

- Use of anti-inflammatory drugs, corticosteroids, immunosuppressants, analgesics, antidiabetics or bisphosphonates regularly or recently.

- Pregnant or breastfeeding women

- Chronic disorders, and history of allergies or adverse effects associated with the medications that were used.

- Completely edentulous patients (absence of erupted teeth).

- Patients who refused to participate in the study, who did not wish to sign the informed consent and those who did not allow follow-up as well as the collection of samples.

B. Sample

The aim is to include and study 30 individuals with an indication for peri-implant treatment in implants, in a healthy state (Category I and II of the ASA) who attend the Department of Oral and Maxillofacial Medicine and Surgery of the Faculty of Dentistry of the Complutense University of Madrid.

Patients will be randomized by computer into a melatonin treatment group or a control group.

6.2. HUMAN RESOURCES

All procedures, from diagnosis, treatment of patients, obtaining samples and complementary tests, as well as sample processing and statistical analysis will be carried out by the doctoral student.

For the supervision and advice of the Research project in the most specific phases, the following qualified personnel will be required:

A biochemist from the Clinical Analysis Service of the Complutense University of Madrid who is able to process and analyze the crevicular fluid samples obtained according to the laboratory techniques indicated for the established biochemical parameters.

A Data Analyst specializing in Biostatistics to apply appropriate statistical formulas to the data obtained, ensuring the objectives of the study.

6.3. PHARMACOLOGICAL MATERIAL

Pure melatonin without excipients: 1.9 mg of melatonin powder, *Farmacia La Botica de Argensola (Madrid, Spain).*

Anesthesia carpules: articaine 4% with adrenaline 1:100,000.

6.4. SURGICAL MATERIAL

Carpule-type metal anesthesia syringes and disposable needles.

Syndesmototomes and Bard-Parker type scalpels with No. 15 blades.

Straight handpiece with saline irrigation and round steel burs.

Surgical aspirators.

Ultrasound.

Stainless steel periodontal curettes.

Periodontal probes.

Langenbeck type retractors, straight and curved cutting scissors, Adson forceps, curved mosquito forceps and Mayo needle holders.

Pre-mounted atraumatic suture needle and 4/0 monofilament silk thread on 3/8 semicircular needle.

6.5. MATERIAL FOR SAMPLE ANALYSIS

Sterile paper points

Perio-paper strips

Sterile transport media

Human IL-1ÿ, 2, 6 ELISA BLK 4-213 and BLK 4-231TEN Kit 79010055 (LETI Laboratories, Barcelona, Spain).

Microbiological culture Petri dishes.

Graduated pipettes of 5 and 10 ml, and tips.

Nuaire Freezer -85ºC Ultralow freezer.

Ivymen System 2100-C ELISA plate reader.

Periotest reader for Perio-paper samples.

SPSS computer program (Static Package for the Social Sciences, version 21.0, SPSS Inc., Chicago, Ill., USA).

7. METHODS

7.1. INTERVENTION AND STUDY GROUPS

The clinical history used in the Department of Stomatology III of the Faculty of Dentistry will be taken first. The informed consent of the patients for the surgical intervention will be obtained, as well as for the application of melatonin and the obtaining of samples of crevicular fluid and digital images.

A complete peri-implant diagnosis will be performed, with peri-implant probing and recording of plaque and bleeding indices. A sample of crevicular fluid will be obtained from two implants for each patient and will be coded so that it cannot be known.
A prior radiological study will be performed on the same day of the intervention. The inclusion of the patient in the melatonin treatment group or in the control group will be randomized by computer, assigning a different code to the cases of application of melatonin and to the control cases that will be unknown to the patient and during the data analysis. The peri-implant treatment and mechanical debridement will be performed in all cases under conventional local anesthesia. The procedure will follow the usual sequence in all cases. Surgical access will be made through an intrasulcular and linear incision, and the detachment of a mucoperiosteal flap. The peri-implant treatment will be performed with an ultrasound tip under irrigation, complemented by the use of curettes if necessary. Subsequently, in the cases of the corresponding group, melatonin (1.9 mg) will be applied to the peri-implant bed.

sutured with 4/0 monofilament silk on a pre-mounted 3/8 semicircular needle.

All patients will be prescribed the same pharmacological therapy consisting of: Paracetamol 1000 mg 1 tablet/8 hours (in case of pain).

After 7 days, the healing process will be reviewed and the sutures will be removed.
New samples of crevicular fluid will be taken from the implants in a coded manner. After 21 days, a new complete peri-implant record will be made with a new crevicular fluid sample. After 60 days, a new radiological study and a complete peri-implant record and crevicular fluid sample will be performed.

All patients will be provided with a 24-hour telephone number to monitor their postoperative status and progress. Assessment of the patient's perception of inflammation and subjective improvement will be carried out through communication with the patient. In addition, the occurrence of adverse events will be recorded for a period of two months after treatment.

All clinical information recorded, as well as the information required to carry out the study, will be treated in such a way that the patient cannot be identified.
To do this, a history number and a case number will be assigned to each patient, without using any information that would allow them to be identified.

7.2. SAMPLE COLLECTION

Enzyme-linked immunosorbent assay (ELISA)

The first sample of crevicular fluid will be obtained by direct extraction from the peri-implant groove in the diagnostic phase. The second sample of crevicular fluid will be obtained 7 days after the intervention. Subsequently,

Crevicular fluid samples at 21 days and 60 days after treatment.

Crevicular fluid will be obtained using Prio-paper strips. The sample will be stored in sterile 13x75mm x 2 ml BD Vacutainer® tubes.

The samples will be transported immediately after collection to the Research Laboratory of the Faculty of Dentistry of the Complutense University of Madrid. The samples will not be treated with any additives, so that 60-90 minutes after collection they will be processed using the Perio-Tron device for the analysis of interleukins.

The samples will be placed at room temperature at the time the determinations are carried out following the instructions of the kit used.

The determinations will be read using the ELISA reader (Ivymen System 2100-C).

Microbiological analysis

The first sample of crevicular fluid will be obtained by direct extraction from the peri-implant sulcus during the diagnostic phase. The second sample of crevicular fluid will be obtained 7 days after the intervention. Subsequently, crevicular fluid samples will be obtained at 21 days and 60 days after treatment.

Crevicular fluid will be obtained using sterile paper points. These will be transferred to a vial of reduced transport liquid, which allows the anaerobic state of the samples to be preserved. The samples will be transported immediately after obtaining them to the Research Laboratory of the Faculty of Dentistry of the Complutense University of Madrid.

The samples are then dispersed, diluted and inoculated into different selective and enriched culture media. The plates are incubated in the appropriate atmosphere for the required time, at which time the plates are examined for morphologically suspicious colonies.

They are subsequently isolated and identified using microscopic and biochemical techniques.

. **Radiographic evaluation**

To obtain the radiographic images, periapical radiographs will be taken using a parallelizing device so that they are always taken in 14

orthogonal position at the diagnostic visit, at 7 days, at 21 days and at 60 days. Panoramic radiographs will be taken at the diagnostic appointment and at 60 days.

The evaluation of the peri-implant crestal bone level will be carried out by evolutionary comparison of the bone level with respect to the implant threads.
prior to treatment, superimposing the x-rays taken subsequently in subsequent visits.

The results obtained will be compared, contrasting the levels of change in patients in whom melatonin is administered versus control patients.

7.3. ANALYSIS

The processing and obtaining of results from crevicular fluid samples will be
carried out by the doctoral student at the Research Laboratory of the Faculty of Dentistry of the Complutense University of Madrid.

The statistical processing of the results obtained will be carried out by the doctoral student under the supervision of a Data Analyst specialized in Biostatistics.

Data will be analyzed using the computer program SPSS (Statistical Package for the Social Sciences, version 21.0, SPSS Inc., Chicago, Ill., USA).

Variables

The independent variables studied will be the characteristics in which the objects of study differ, which can be specified as the case group, treated with melatonin, compared to the control group, treated conventionally.

Dependent variables are those whose behavior is explained or predicted by one or more independent variables. Dependent variables may be considered to be the concentration of interleukin-1ÿ, 2 and 6, microbiological culture determined in crevicular fluid samples, probing depth, as well as the amount of bone determined in diagnostic imaging tests.

8. LIMITATIONS

This research protocol presents a series of limitations that are detailed below.

First, since this is a pilot study, the patient sample may not be enough to assume the results as sufficient scientific evidence.

Despite the scientific evidence available, there are no previous studies on the use of melatonin in a study model such as the one proposed. Nor are there any studies using the doses chosen in the present research protocol.

There is controversy regarding the effects of melatonin described. It is described as a pleiotropic molecule whose effects depend on the previous state of the receptors. In this regard, there are few previous studies detailing the properties of this hormone in situations such as those raised in the present study locally in patients. On the other hand, systemic changes in the individual, as well as their habits, can locally influence the study variables.

Although the present study model is considered ideal for assessing the properties of melatonin, there may be intersubject and even intrasubject differences due to peri-implant bone defects.

As regards methodology, the laboratory processes and the measurement and comparison of radiographic images will be carried out by qualified personnel. However, the possibility of individual variability must be taken into account.

9. SCHEDULE

	SEPT-NOV 2018	DEC 2018-FEB 2019	SEA-MAY 2019	APR-JUL 2019	SEPT-NOV 2019	DEC 2019-FEB 2020	SEA-MAY 2019	JUN-SEPT 2019	OCT-DEC 2020	JAN-MAR 2021	APR-JUN 2021
Preparation and correction of the Protocol											
Drug Agency Approval Request											
Ethics Committee Approval Request											
Validation of methodology and drugs											
Purchase of kits and pharmacological products. Testing of materials											
Patient recruitment: Intervention, re-evaluation and second re-evaluation											
Sample processing											
Measurement of radiographic tests											
Data organization											
Interpretation of results											
Writing the Manuscript-Thesis											
Preparation of the presentation											
Dissemination of results											

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ANNEX I

INFORMATION SHEET FOR POTENTIAL PARTICIPANTS (RD 561/93)

Aim:

The Research Project "EFFECT OF MELATONIN IN THE TREATMENT OF PERI-IMPLANT DISEASES" aims to provide an effective alternative in the treatment of peri-implant bone loss by measuring biochemical and microbiological parameters obtained from crevicular fluid samples. It also aims to demonstrate its usefulness in bone regeneration, obtaining bone of adequate volume and density in a shorter period of time through digital comparison of images obtained by orthopantomography and periapical series.

Methodology used:

Phase IV randomized clinical trial study with analysis of biological variables.
Measurement of certain molecules indicating inflammation and infection in crevicular fluid using biochemical enzyme immunoassay and culture techniques and measurement of bone quantity using a computerized system for counting surfaces obtained in an orthopantomographic image and periapical series, comparing the measurements with those obtained from the control.

Expected benefits for the patient or society:

The benefit is obtaining a natural substance that can be used in the same surgical session to reduce inflammation and the risk of infection from the very beginning, as well as promoting the formation of adequate and sufficient bone in less time.

Discomforts and risks arising from the study:

Two samples of crevicular fluid will need to be obtained from each peri-implant groove, so two samples will be processed from each side immediately before surgery and two more from each side at 7, 21 and 60 days after surgery.

Two digital panoramic images will also be required, obtained in two visits and a periapical series in the four scheduled visits. The first panoramic image will be performed prior to the treatment of peri-implant bone loss, on the same day of the intervention. The second will be performed 60 days later during the review of the healing process.

The only inconvenience is that of attending the check-up, since obtaining samples of crevicular fluid is a procedure that is performed without discomfort for the patient.

Possible adverse events:

The only adverse effects are those inherent to the surgical procedure for treating peri-implant bone loss. Sometimes it is not possible to maintain implants affected by this disease and they may therefore be removed.

Participation is voluntary, and patients may withdraw from the study at any time without altering the doctor-patient relationship or harming their treatment.

Data protection:

Your personal data will be accessed, with your prior consent, by healthcare professionals who are involved in the treatment of peri-implant bone loss and in obtaining crevicular fluid samples in the Department of Stomatology III (Buccofacial Medicine and Surgery) of the Faculty of Dentistry at the Complutense University of Madrid, coordinated by Dr. Carlos Cobo. The data collected in the Medical History, as well as the results of the study of the saliva samples and the panoramic X-ray images, will be separated from your personal identity, assigning the same code to the Medical History and the results of the study, so that it will not be possible for research staff outside the Department of Stomatology III (Buccofacial Medicine and Surgery) of the Faculty of Dentistry to associate them without express authorization.

Method of financial compensation and treatment in the event of damage or injury due to participation in the trial, as set out in the Medicines Law:

There is no financial compensation.

Researcher responsible for the Research Project and for informing the subject and answering his/her questions and doubts, and how to contact him/her in case of emergency:

The principal investigator of the Research Project is Dr. Carlos Cobo Vázquez, from the Faculty of Dentistry of the Complutense University of Madrid. In the Department of Stomatology III (Medicine and Oral and Facial Surgery), the coordinating investigator is Dr. Juan López-Quiles Martínez, whose contact telephone number is 91 394 1964.

ANNEX II

INFORMED CONSENTS FOR POTENTIAL PARTICIPANTS

STATEMENT OF INFORMED CONSENT

Mr./ Mrs....., aged ..... years and with DNI number....., declares that he/she has been informed by..... about the benefits that could be obtained from obtaining samples of peri-implant crevicular fluid from each side to cover the objectives of the Research Project entitled **"EFFECT OF MELATONIN IN THE TREATMENT OF DISEASES**

"PERIIMPLANTARIAS", carried out by researchers from the Department of Stomatology III (Buccofacial Medicine and Surgery) of the Faculty of Dentistry of the Complutense University of Madrid. The purpose of this study is to obtain an effective alternative in the treatment of inflammation and peri-implant bone loss.

produced by peri-implant infection with utility in early bone regeneration, obtaining bone of adequate volume and density in a shorter period of time.

I have been informed of the possible harm that obtaining crevicular fluid samples may have on my well-being and health, which are attributable to a routine test.

I have also been informed that my personal data will be protected in accordance with the provisions of Organic Law 15/1999, of December 13, on the Protection of Personal Data. The samples will be anonymized and the necessary clinical data will be requested from me at the time of the intervention and I authorize that they be consulted in my Medical History by the Doctor of the Research Team of the Health Center. Only the part of the Medical History whose data is relevant to the study will be accessed, and in any case, the Doctors of the Research Team undertake to maintain the strictest confidentiality.

Taking this into consideration, I GRANT my CONSENT for this extraction to take place and be used to meet the objectives specified in the project.

Madrid, a of of 2018.

Signed Mr/Mrs. Signed Mr./Mrs. Optional

(Patient) (Guardian, in case of incapacity)

STATEMENT OF INFORMED CONSENT

Mr./ Mrs....., aged ..... years and with DNI number....., declares that he/she has been informed by..... about the benefits that could be derived from performing two digital orthopantomographies and periapical series.
to cover the objectives of the Research Project entitled **"EFFECT OF MELATONIN IN THE TREATMENT OF PERI-IMPLANT DISEASES** ", carried out by researchers from the Department of Stomatology III (Buccofacial Medicine and Surgery) of the Faculty of Dentistry of the Complutense University of Madrid. The purpose of this study is to obtain an effective alternative in the treatment of inflammation and peri-implant bone loss caused by peri-implant infection with utility in early bone regeneration, obtaining a bone of adequate volume and density in a shorter period of time.

I have been informed of the possible harm that performing two digital orthopantomographies may have on my well-being and health, which are attributable to a routine examination.

I have also been informed that my personal data will be protected in accordance with the provisions of Organic Law 15/1999, of December 13, on the Protection of Personal Data. The images will be anonymized and the necessary clinical data will be requested from me at the time of the intervention and I authorize that they be consulted in my Medical History by the Doctor of the Research Team of the Health Center. Only the part of the Medical History whose data is relevant to the study will be accessed, and in any case, the Doctors of the Research Team undertake to maintain the strictest confidentiality.

Taking this into consideration, I GRANT my CONSENT for this test to take place and be used to meet the objectives specified in the project.

Madrid, a of of 2018.

Signed Mr/Mrs. Signed Mr./Mrs. Optional

(Patient) (Guardian, in case of incapacity)