Immediate loading-A histological study in humans.

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Running Head: Immediate and delayed loading

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Abstract

Objective: - To evaluate histologically the influence of the functional load on implants immediately loaded

Material and methods: 12 patients with edentulous alveolar crests in the distal portion of the arches will be included in the study. At least two implant sites will be identified in each patient and will be sub-prepared with drills or sonic instruments to obtain a high degree of torque to ensure optimum primary stability. At least one site will be used as a test and one as a control where experimental mini-implants will be installed. Test sites will be loaded immediately. The control sites will remain unloaded. After 2 months, mini-implants will be recovered as planned. Reconstructive surgical procedures will be applied at the biopsy sites, if necessary, using autologous bone or bone substitutes and membranes. Standard implants will be installed during the same surgical session and, after 2 months, the planned prosthesis will be performed. Patients will be followed for at least 2 years for evaluation and intervention, if necessary.

Biopsies will be included in resin to obtain wear cuts for histomorphometric analysis. The bone histomorphometry will be evaluated and statistical analysis will be carried out.

Introducción

It has become evident a growing concern related to the possibility of shortening the healing period in implant-prosthetic rehabilitation and numerous clinical studies have reported on the results of the immediate loading of implants in various clinical situations (Esposito et al., 2009; et al., 2010). Clinical studies (Calandriello et al 2003, Glauser et al 2005, Lindeboom et al 2006, Donati et al 2008) demonstrated that the immediate functional load of implants placed with the conventional technique and with sufficient primary stability can be considered a valid therapeutic alternative in the replacement of unique teeth.

The healing around the loaded implants has been immediately examined in several animal experiments. Romanos et al. (2002) placed three implants on each side of the jaw in six dogs. The implants at the test sites were loaded immediately after the installation, while the implants from the control sites were left in a submerged position for 3 months. The test and control implants were exposed to functional load for 3 months. It was reported that the percentage of bone-implant contact (BIC) did not differ in the test sites with respect to the controls, while the bone density in the area between threads was significantly larger around the test implants than in the controls. Bousdras et al. (2007) analyzed bone reactions adjacent to simple implants exposed to immediate loading for 10 weeks in a porcine model. Nine of the twelve implants exposed to functional loading failed to integrate. In the remaining sites, the histological analysis showed that there was no difference in the bone-implant contact percentage (BIC%) and in the bone density between the test implants and the controls. Lee et al. (2009) evaluated the effect of immediate loading on the BIC% in three dogs. Biopsies were obtained 2 months after implant placement. A single cut was taken into account for each implant for histological analysis. No difference was observed between the test and control implants in terms of BIC%. In another experiment in dogs (Rea et al., 2014), in which the results of implants installed with different torques and immediately

loaded or left unloaded were studied, statistically significant differences were found, both for the BIC% and for the Percentage of Bone density, especially when a low insertion torque (about 30Ncm) was used.

Human biopsies have often been used to study hard and soft tissue reactions around experimental implants with small dimensions that are regularly used in regular dental implants (Lang et al., 2011; Cecchinato et al., 2012). In such studies, the experimental implants were removed using a trephine.

Human histological studies on immediately loaded implants have reported conflicting results in relation to the degree of bone-implant contact (BIC%) in relation to conventionally loaded implants (Degidi et al 2003b, 2009, Rocci et al 2003, Romanos 2009). Such studies were also based on few samples recovered from different subjects and the question whether the immediate functional load is beneficial or prejudicial remains unresolved.

In a study conducted by Rocci et al. (2003) five subjects received implants-supported prostheses in the posterior part of the jaw. In one subject, two implants were exposed to immediate loading, while seven implants in four subjects were loaded after 2 months of healing without disturbance. The two loaded implants were immediately recovered together with the adjacent peri-implant bone after 9 months of function, while biopsies of the remaining remnant sites were obtained after a period between 5 and 7 months. The histological examination showed a higher percentage of bone-implant contact (BIC%) for the loaded implants immediately than for the implants exposed to "delayed loading". Degidi et al. (2003a) collected biopsies from two implant sites exposed to immediate loading in a subject and from an implant site not loaded in a second patient. 6 months after the placement of the implants, both the immediately loaded and the unloaded ones were removed for histological analysis. The results indicated that the loaded implants immediately had a higher BIC% than the controls.

Romanos et al. (2005) reported in a series of cases about immediate loading and human biopsies. Of 29 implant biopsies obtained for histological analysis, one site represented a "control" unit that was not exposed to immediate loading. Biopsies were obtained after 2-10 months of healing / loading. It was reported that the percentage of bone-implant contact (BIC%) varied between 54% and 80%. In a simple cut of a test site and a control of implants obtained from a subject, the BIC% was higher in the test site than in the control. Degidi et al. (2009) collected biopsies of an implant loaded immediately in each of two patients and a control implant in each of the other two subjects. The results of the histological analysis indicated a higher BIC% in the test sites than in the controls.

In addition, no differences were observed between the test and control implants in terms of percentage of bone-implant contact in a histological study in humans (Donati et al., 2013) in which the implants were immediately loaded or left unloaded. Biopsies were obtained after 1 and 3 months of healing.

Due to the lack of clear evidence due to small samples or methodological errors, there is a need to obtain more information about the histological results around the immediately loaded implants.

Therefore, the purpose of the present study is to evaluate histologically the influence of functional load on implants, both immediate and delayed.

Material and Methods

Study Population

Adult subjects (≥25 years of age) with the need of at least two implants to replace teeth of the mandible will be included. Natural antagonist teeth have to be present both at test and control sites to guarantee occlusal contacts.

The exclusion criteria will be the following: (i) untreated rampant caries or uncontrolled periodontal disease of the remaining teeth; (ii) diabetes not controlled diabetes or any other systemic or local disease that could compromise postoperative healing and / or osseointegration; (iii) need for systemic corticosteroids or any other medication that could compromise postoperative healing and / or osseointegration; (iv) inability or unwillingness to return to follow-up not likely to be able to comply with the study procedures according to the judgment of the investigators; (v) ≥10 cigarettes per day; (VI) pregnancy.

Randomization

Each patient will receive two mini-implants. The two recipient sites will be selected prior the surgery, and the type of load will be randomly decided. A researcher (DB), neither involved in the selection of the patients nor in the surgical and prosthetic treatment, will carried out electronically the randomization (randomization.com). Sealed opaque envelopes were prepared and opened at the time of surgery.

Experimental procedures

Patients will be carefully selected and their oral status assessed. Patients with edentulous areas that allow the installation of at least 2 implants will be included in the study. Adequate oral prophylaxis and treatments, including extractions of lost teeth, will be performed on all

patients. After 3 months of extractions, the areas will be identified and local anesthesia will be provided.

Patients will be assigned to immediate functional loading or no load groups. In each patient, edentulous areas with adequate thickness of the alveolar bone crest will be identified for implant installation. All implant recipient sites will be sub-prepared to allow good stability of the implants. Experimental mini-implants (Sweden & Martina, Due Carrare, Padova, Italy), 3.5 mm in diameter and with an intraosseous portion of 4 mm in length will be installed.

Immediate loading group - A prosthetic component in resin will be applied in the same session over the trial implants. A control of the occlusion will be carefully performed. Control implants will receive a healing screw and will remain unloaded.

Patients will receive antibiotics (e.g., 1 g of amoxicillin twice a day for 5 days), anti-inflammatories (e.g., 600 mg of Ibuprofen) as needed, and chlorhexidine (0.2%) twice a day for two weeks. An inspection of the wounds will be carried out regularly in search of clinical signs of complication.

Retrieval of mini-implants

After two months in both groups, the mini-implants will be removed. After the application of local anesthesia, mini-invasive flaps will be raised around the mini-implants, leaving a small portion of soft tissue on the buccal side using a sonic device (Sonosurgery® Air Power, TKD, Calenzano, FI, Italy) , and a specific insert (Komet, Lemgo, Germany) as shown in a recent thesis (Ferri et al., 2014). Subsequently, a cut will be made at the base of the implant to release the biopsy. This system will leave smaller donor sites (Ferri et al., 2014) compared to those produced by the use of a trephine (Donati et al., 2013) and the biopsies will have a higher quality (Ferri et al., 2014; Lang et al., 2011). Bosshardt et al., 2011). However, if necessary, a trephine of approximately 4 mm in diameter at a depth of 4 mm

should be used. The biopsies will be preserved and fixed in 4% formaldehyde solution and processed for histomorphometric analysis.

Installation of standard implants

In the same surgical removal of the implants, standard container sites will be prepared after the flaps are lifted and standard implants of a suitable length and diameter will be placed (Sweden & Martina, Due Carrare, Padova, Italy) in the same position than in the biopsy or, possibly in another position adequate to obtain the planned prosthetic restoration. In both cases, the biopsy region may require limited regenerative procedures. In this case, deproteinized bone mineral or autologous bone obtained from adjacent regions will be used, applying a membrane to protect the region. Submerged or non-submerged healing of standard implants will be allowed, depending on the clinical situation. The wounds will be closed with resorbable sutures. Patients will receive antibiotics (e.g. 1 g amoxicillin twice a day for 5 days) and anti-inflammatories (e.g. 600 mg Ibuprofen) as needed and chlorhexidine (0.2%) swish twice a day for 2 weeks. An inspection of the wounds will be carried out regularly in search of clinical signs of complications.

After 2 months of healing, the final restoration of the regions used for the experimental purposes will be carried out. Patients will be included in a follow-up of at least 2 years. The possible biological and technical complications of prosthetic rehabilitation will be treated.

Comments on the selection and treatment of patients

An effort will be made in the recruitment of patients with totally edentulous jaws or large edentulous regions to allow the use of different areas for the installation of mini-implants or standard implants.

Histological preparation

The individual blocks containing the implant surrounded by hard tissues will be fixed in 4% formaldehyde solution followed by dehydration in graduated series of ethanol and finally included in resin (LR White® hard grade, London Resin Company Ltd, Berkshire, UK). The blocks will be cut using a diamond band attached to precision cutting equipment (Exakt®, Apparatebau, Norderstedt, Germany) and then reduced to a thickness of approximately 50µm using wear equipment (Exakt®, Apparatebau, Norderstedt, Germany).

For each block, one or two histological slides will be obtained from the central part of the implants and stained by means of staining of Stevenel's Blue and Alizarin Red or Toluidine Blue and examined in a standard optical microscope for histometric analysis.

Histometric evaluation.

In an Eclipse Ci microscope (Nikon Corporation, Tokyo, Japan), equipped with a digital video camera (Digital Sight DS-2Mv, Nikon Corporation, Tokyo, Japan) connected to a computer, the percentages of mineralized and non-mineralized tissues in contact with the surface of the implant between the most coronal bone to implant contact (B) and the apex of the implant (A) will be measured.

The percentage of mineralized and non-mineralized tissues will also be in an area between B and A. For this purpose, a region will be superimposed on the region to be examined.

Statistic analysis.

In an experimental study in dogs (Rea et al., 2014), which compared the results of the implants installed with different torques and loaded immediately or left unloaded, statistically significant differences were found both in the percentage of bone-implant contact (BIC%) as in Percentage of bone density, especially when a low insertion torque (~30Ncm) was applied. Six animals were used in the study. Considering the high heterogeneity of the

human sample, it seems reasonable to include 10-12 patients per group in the present study, with the possibility of extending it to 15 if necessary and possible.

The primary variable will be bone-to-implant contact and the secondary variable will be bone density around the implant.

The differences between the test sites and the controls will be analyzed using the Wilcoxon test for paired observations.

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The participants in the study declare that there are no conflicts of interest.

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