12.6 Osseointegration of Dental Implants in a Human Model

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12.6.1 Background and Scientific Rationale

Implant placement into alveolar bone induces a cascade of healing events resulting in direct bone contact with the implant surface. Direct bone-to-implant contact (BIC) was first described by Per-Ingvar Brånemark and coworkers (Brånemark *et al*., 1969, 1977) and histologically demonstrated for the first time by André Schroeder and coworkers as "functional ankylosis" (Schroeder *et al*., 1976, 1978, 1981). Osseointegration is defined as "a direct, structural and functional connection between ordered, living bone and the surface of a load-bearing implant" (Listgarten *et al*., 1991). Thus, quantification of osseointegration is performed in histological sections by measuring the proportion of bone-to-implant contact (BIC) along the external implant surface. To create true BIC values, the analysis needs to be performed along the intact tissue-implant interface and without the presence of aberrations. Therefore, micro-computed tomography (μ-CT) and histomorphometrical analysis of tissues where the implant had been removed prior to histological processing may be considered as inadequate (Butz *et al*., 2006). Indeed, in most studies where the BIC was analyzed, ground sections (i.e., histological sections of undecalcified tissues) were performed.

A number of studies show that titanium implants with various surface characteristics do osseointegrate in human jawbone as demonstrated by histomorphometry. Implants retrieved from humans represent a very inhomogeneous group of different implant types explanted for various reasons including implant failure. Implants retrieved 2 to 10 months following installation without bone grafting showed BIC values between 7% and 100% (Piattelli *et al*., 1998, Wilson *et al*., 1998, 2003; Ivanoff *et al*., 2001, 2003; Romanos *et al*., 2005; Grassi *et al*., 2007). The BIC values largely depended on location, implant design, and implant surface characteristics (i.e., topography and chemistry). In single dental implants retrieved from humans after 4, 5, 10, and 12 years in function, the BIC amounted to 78.1%, 83.2%, 77.4%, and 94.1%, respectively (Schenk and Buser, 1998). In one case, four bar-connected titanium plasma-sprayed (TPS) implants retrieved from a 95-year old patient revealed a mean BIC of 76.4% after 12 years of functional load (Ledermann *et al*., 1998). These studies clearly showed that dental implants installed in jawbone of humans become osseointegrated and that osseointegration of functionally loaded dental implants generally lasts for many years. Knowledge on the sequence of healing events leading to osseointegration of dental implants in humans, however, is sparse. A recent series of studies aimed at evaluating histological and molecular levels the rate and degree of osseointegration at chemically modified at moderately rough, hydrophilic and hydrophobic implant surfaces during early phases of healing in a human model (Bosshardt *et al*., 2011; Donos *et al*., 2011; Ivanovski *et al*., 2011; Lang *et al*., 2011). The findings of these studies (Bosshardt *et al*., 2011; Lang *et al*., 2011) indicated that implant placement into alveolar bone induces a cascade of healing events starting with clot formation and continuing with bone maturation in contact with the implant surface. From a molecular point of view, osseointegration is associated with a decrease in inflammation and an increase in osteogenesis-, angiogenesis- and neurogenesis-associated gene expression during the early stages of wound healing (Donos *et al*., 2011; Ivanovski *et al*., 2011).

Although important findings on the interaction of individual cell types and titanium surfaces may be obtained from *in vitro* studies, they do not fully reflect the complex mechanisms during tissue integration of implants *in vivo*. Based on the fact that wound healing and tissue formation, especially bone formation, may occur at a higher speed

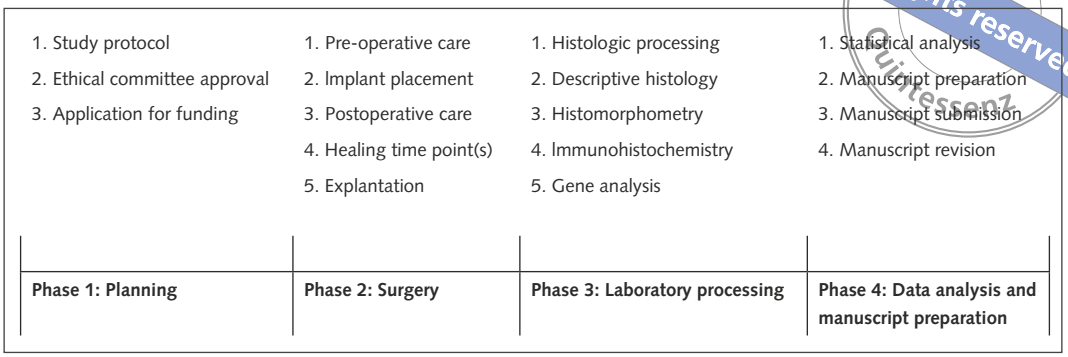

Fig 12.6-1 The four phases of a clinical study evaluating osseointegration of dental implants in a human model.

in experimental animals compared with humans, findings from animal experiments may not be transferred directly to the human situation without adaptation.

Hence, it is the aim of this chapter to summarize the steps necessary to conduct a study on osseointegration of dental implants in humans.

12.6.2 Timeline of the Study (Fig 12.6-1)

Studies evaluating osseointegration in humans should be divided into four phases:

1. Planning Phase

Preparation of the study protocol, including a clear statement of the scientific hypothesis, is to be carried out. In order to receive financial support, the principal investigator of the study should consider submission of the protocol to institutions and foundations. A clinical study should only be initiated after the protocol has been submitted to and approved by the appropriate ethical or human subjects committee.

2. Surgical Phase

Preoperative care, implant placement, postoperative care, healing time-point(s) and explantation.

3. Laboratory Phase

The events leading to osseointegration should be analyzed by a descriptive analysis of histological sections at different time-points following implant installation, and by histomorphometric measurements of the BIC with respect to implant landmarks. Moreover, immunohistochemical and gene analysis techniques may be applied.

4. Data Analysis and Preparation of the Manuscript

The timeframe for performing a clinical study may depend on the number of subjects included, the healing time-points chosen and the laboratory techniques applied. One to 2 years may be required, from the planning phase to the final version of the manuscript.

12.6.3 The Four Phases

1. Planning Phase

Subject Sample Inclusion Criteria

Subjects fulfilling the following inclusion criteria should be enrolled:

- age ≥ 18 years
- absence of relevant medical conditions
- absence of regular intake of medications affecting bone metabolism (e.g., bisphosphonates, corticosteroids)
- smoking ≤ 5 cigarettes/day
- periodontal health or treated periodontal conditions
- absence of mandibular third molars or healed extraction sockets after third molar extraction

- presence of sufficient parent bone volume in the retromolar area for experimental implant installation
- signed informed consent.

Exclusion Criteria

Subjects should not be enrolled, if the following conditions are present:

- uncontrolled medical conditions
- untreated periodontal conditions
- contraindications for oral surgical procedures
- pregnant and lactating females
- unwillingness to comply with study protocol.

Power Calculation

A standard normal distribution should be assumed. The probability of a type I error will be set at $\alpha = 0.05$ and of a type II error at $\beta = 0.20$ in order to achieve a study power of 80%. The standard deviations reported in previous similar experiments should be used as a reference.

Mean values and standard deviations should be calculated for each variable and group. Normal distribution of the data should be tested. Depending on the data distribution, either non-parametric or parametric tests should be applied in the statistical analysis.

Experimental Implant Configuration

The implants used should have a geometry corresponding to that of a solid screw implant for clinical use and be made of commercially pure titanium, titanium-zirconia alloys or zirconia. A U-shaped circumferential trough should be prepared within the thread region of the endosseous portion of the implant leaving the tip of the thread untouched. This would create a well-defined wound compartment with landmarks that can be identified after explantation and histological processing.

Experimental Implant Surface Roughness and Chemistry

Implants with a micro-rough surface may be tested. It should be verified that the implant surfaces correspond to their counterparts of commercially available implants with respect to Sa and Ra values.

Moreover, implant surface wettability measured with dynamic contact angles (DCA) should be assessed. This would allow the distinction between hydrophobic and hydrophilic implant surfaces.

Healing Time-points

Early healing periods ranging from 7 days up to 6 weeks after implant installation should be planned. Healing periods after 6 weeks following implant installation may no longer provide the dynamic processes during tissue integration. Rather, they represent a stage of homeostasis in which remodeling processes may take place.

Test Groups

A split-mouth study design testing devices with two different materials or surface characteristics in the same subject may be planned.

2. Surgical Phase

Preoperative Care

In order to avoid an accidental perforation of the lingual cortical plate in the retromolar area, a three-dimensional image may be indicated. Peri-operative complications (i.e., events occurring during the surgical procedure itself or during the early stages of wound healing) should be avoided by enrolling only subjects fulfilling the inclusion criteria.

The use of prophylactic systemic antibiotics is not indicated for straightforward implant installation.

Surgical Procedure

The same surgical procedures should be performed on the test and on the control sides of human volunteers. Following local anesthesia, a mucoperiosteal flap is raised in the retromolar areas, and the recipient sites are marked on the alveolar crest. Following this, the implant recipient sites should be prepared to their final diameter. The experimental implants should be placed in such a position that the most coronal aspect of

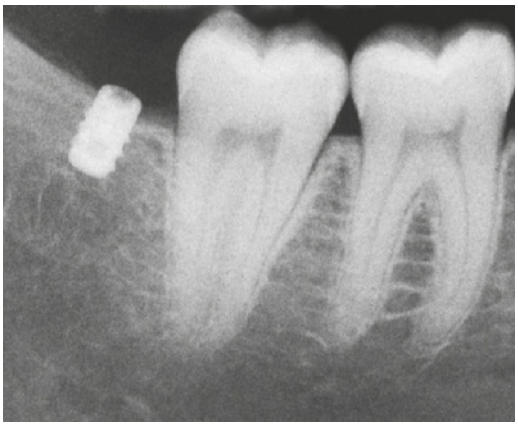

Fig 12.6-2 Periapical radiograph taken immediately after experimental implant placement in the retromolar area of a human volunteer. A safe distance to anatomic structures such as the inferior alveolar nerve and the adjacent tooth is respected.

them will be at the level of the alveolar crest. After checking for primary stability, a cover screw should be placed on top of each experimental device and the flaps sutured to obtain primary wound closure (i.e., submerged healing). A control radiograph should be taken at this time (Fig 12.6-2).

Postoperative Care

Subjects should be advised to follow carefully the postoperative instructions. A rinse with 0.1 to 0.2% chlorhexidine digluconate twice daily should be prescribed for a period of 14 days. Analgetic medications should be prescribed according to individual needs. The use of postoperative systemic antibiotics is not indicated in routine straightforward implant installation (Tan *et al*., 2013).

The healing process should be assessed on a weekly basis and the sutures removed after 7 days.

At the time of explantation, mucoperiosteal flaps should be raised exposing the test devices and a guiding cylinder should replace the cover screw (Fig 12.6-3). The retrieval of the test devices using a specially designed trephine bur should allow for the harvesting of a tissue collar of approximately 1 mm thickness around the entire circumference of the devices installed (Figs 12.6-4 to 12.6-6). The guiding cylinder is necessary to avoid unforeseen angulation of the explantation trephine slipping towards the device proper.

3. Laboratory Phase

Histological Processing

The specimens should be transported to the laboratory in 4% buffered formalin. The specimens are rinsed in tap water, dehydrated in alcohol and embedded in methylmethacrylate (Schenk *et al*., 1984). Undecalcified ground sections are obtained and stained. Toluidine blue may be chosen for light microscopic analysis.

Histomorphometric Measurements

Descriptive histological analysis of the various tissue components (i.e., old bone, new bone, bone debris and soft tissue) occupying the area adjacent (e.g., 1 mm) to the implant surface should be performed for all samples (Fig 12.6-7).

The following linear measurements of the most central sections of the implant should be performed in order to calculate the percentages of the various tissue components in contact with the implant surface:

- *Primary endpoint:* the amount of newly formed bone in direct contact with the surface of the implant (BIC) should be expressed as percentage of the artifact-free implant-tissue interface (Fig 12.6-8).
- *Secondary endpoints:* the amount of old bone (OB), bone debris (BD) and soft tissue (ST) in contact with the implant surface should be expressed as percentage of the artifact-free implant-tissue interface (Fig 12.6-8).

Additional Observations and Measurements

- *Immunohistochemistry:* the use of immunohistochemical techniques may be advocated to detect the expression of markers of bone metabolism involved in osseointegration. Such

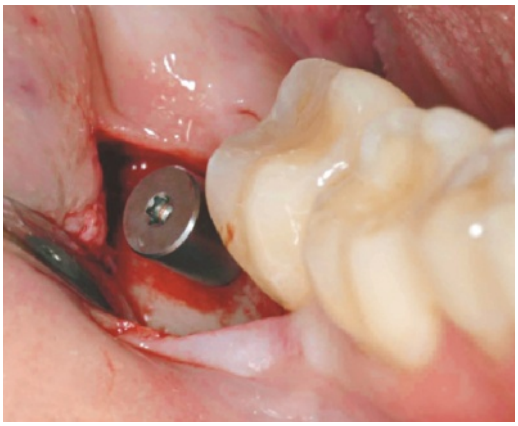

Fig 12.6-3 Guiding cylinder mounted on the experimental implant. The trephine bur will be guided during explantation and produce a tissue collar of approximately 1 mm around the implant.

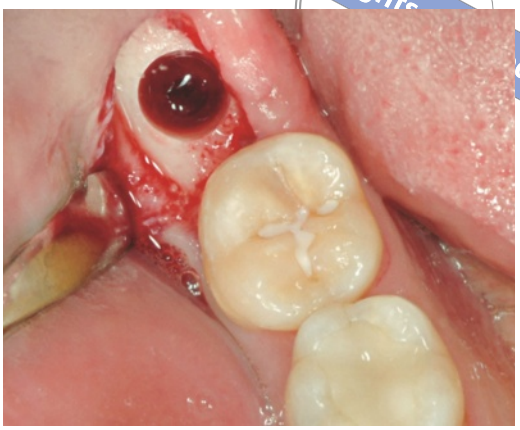

Fig 12.6-4 Explantation wound following removal of the experimental implant. The wound will fill with a coagulum and heal after suturing the flap over the site.

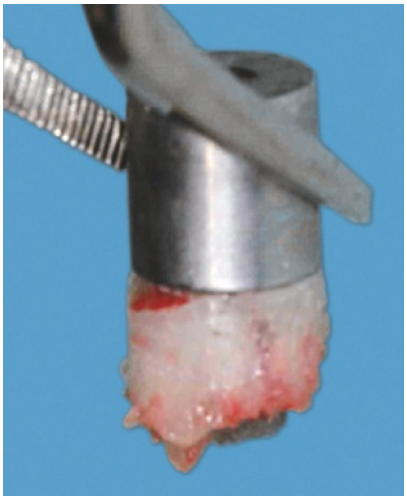

Fig 12.6-5 Guiding cylinder with the intact tissue collar around the experimental implant after explantation.

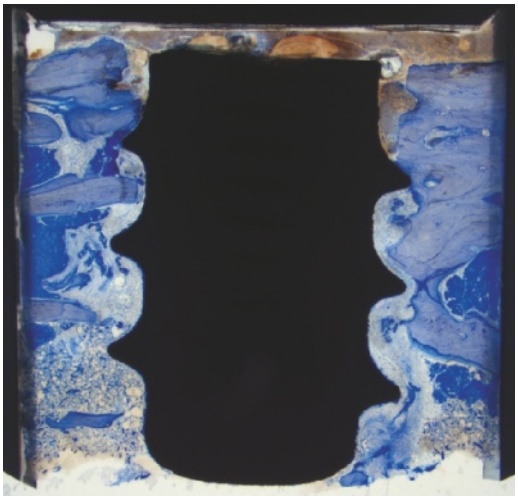

Fig 12.6-6 Longitudinal ground section through an experimental solid screw implant device and trephine bur after a healing period of 14 days. Note the presence of a collar of tissue measuring about 1 mm in width between the implant surface and the trephine. While compact bone is found in contact with the coronal portion of the implant, the circumferential bone in the apical portion is less dense and old bone is not in contact with the implant surface (from Lang *et al*., 2011).

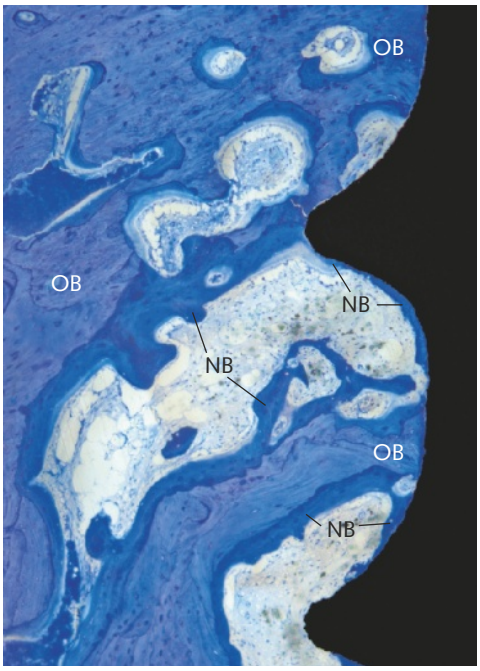

Fig 12.6-7 Osseointegrated experimental implant with a hydrophilic surface 42 days after installation. While some pristine (i.e., old) bone (OB) is still in contact with the implant surface, new bone (NB) is present on old bone and on the implant surface (from Bosshardt *et al.,* 2011).

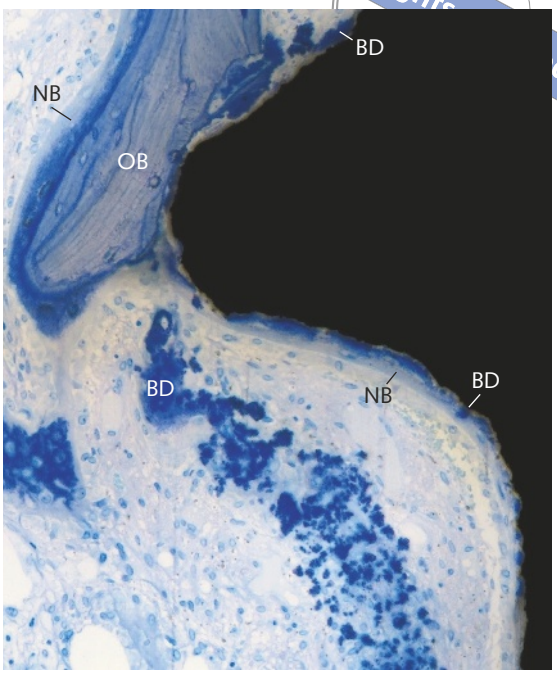

Fig 12.6-8 After a healing period of 7 days, approximately 6 % of a hydrophilic implant surface is covered with new bone (NB). While pristine (i.e., old) bone (OB) is still in contact with the pitch of the implant thread, new bone formation occurs on old bone and on the implant surface and in association with bone debris (BD) found adhering to the implant surface and embedded in the adjacent soft tissue. The new bone mainly consists of osteoid lined by osteoblasts (from Bosshardt *et al*., 2011).

markers may include osteopontin (OPN), osteonectin (ON), bone sialoprotein (BSP), collagen III and integrins.

- *Gene analysis techniques:* gene profiling using microarrays may be used to assess the full genetic profile of a given sample. This technology will allow insight into specific healing-associated genetic pathways leading to osseointegration. These key biological processes include inflammation, angiogenesis, neurogenesis, skeletogenesis, as well as signaling pathways regulated during the early stages of osseointegration. The regulation of these biological processes during the early stages of osseointegration in a human model would provide a unique insight into the cellular and molecular mechanisms associated with bone wound healing in response to the insertion of a titanium dental implant.

Moreover, the combination of histological analysis following the use of established experimental *in vivo* models, coupled with the genetic analysis using microarrays, allows direct correlation between molecular and clinical events during healing. The ultimate goal is to improve the understanding on how and when the pathway of bone healing could be modulated in order to achieve predictable osseointegration at compromised sites.

4. Data Analysis and Preparation of the Manuscript

Before a manuscript draft is prepared, the consultation of a biostatistician may be helpful for the selection of the appropriate statistical tests.

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References

1. Bosshardt DD, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. Clin Oral Implants Res 2011;22:357–364.
2. Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plastic Reconstructive Surg 1969;3:81–100.
3. Brånemark PI, Hansson BO, Adell R, *et al*. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Sc. Scand J Plastic Reconstr Surg 1977;(Suppl)16:1–132.
4. Butz F, Ogawa T, Chang TL, Nishimura I. Three-dimensional bone-implant integration profiling using micro-computed tomography. Int J Oral Maxillofac Implants 2006;21:687–695.
5. Donos N, Hamlet S, Lang NP, *et al*. Gene expression profile of osseointegration of a hydrophilic compared to a hydrophobic microrough implant surface. Clin Oral Implants Res 2011;22:365–372.
6. Grassi S, Piattelli A, Ferrari DS, *et al*. Histologic evaluation of human bone integration on polished and sandblasted acid-etched titanium surfaces in type IV bone. J Oral Implantol 2007;33: 8–12.
7. Ivanoff CJ, Hallgren C, Widmark G, Sennerby L, Wennerberg A (2001). Histologic evaluation of the bone integration of TiO2 blasted and polished titanium microimplants in humans. Clinical Oral Implants Research 12:128-134.
8. Ivanoff CJ, Widmark G, Johansson C, Wennerberg A. Histologic evaluation of bone response to oxidized and polished titanium micro-implants in human jawbone. Int J Oral Maxillofac Implants 2003;18:341–348.
9. Ivanovski S, Hamlet S, Salvi GE, *et al*. Transcriptional profiling of osseointegration in humans. Clin Oral Implants Res 2011;22:373–381.
10. Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. Clin Oral Implants Res 2011;22:349–356.
11. Ledermann PD, Schenk RK, Buser D. Long-lasting osseointegration of immediately loaded, bar-connected TPS screws after 12 years of function: a histologic case report of a 95-year-old patient. Int J Periodontol Restorative Dent 1998;18:552–563.
12. Listgarten MA, Lang NP, Schroeder HE, Schroeder A. Periodontal tissues and their counterparts around endosseous implants. Clin Oral Implants Res 1991;21: 1–19.
13. Piattelli A, Scarano A, Piattelli M. Histologic observations on 230 retrieved dental implants: 8 years' experience (1989-1996). J Periodontol 1998;69:178–184.
14. Romanos GE, Testori T, Degidi M, Piattelli A. Histologic and histomorphometric findings from retrieved, immediately occlusally loaded implants in humans. J Periodontol 2005;76:1823–1832.
15. Schenk RK, Buser D. Osseointegration: a reality. Periodontology 2000 1998;17:22–35.
16. Schenk RK, Olah AJ, Hermann W. Preparation of calcified tissues for light microscopy. In: Dickson GR (ed). Methods of Calcified Tissue Preparation, Vol 1. Amsterdam, Netherlands: Elsevier, 1984:1–56.
17. Schroeder A, Pohler O, Sutter F. Gewebereaktion auf ein Titan-Hohlzylinderimplantat mit titan Spritzschichtoberfläche. Schweizerische Monatsschrift für Zahnheilkunde 1976:86:713–727.
18. Schroeder A, Stich H, Straumann F, Sutter F. The accumulation of osteocementum around a dental implant under physical loading. Schweizerische Monatsschrift für Zahnheilkunde 1978;88:1051–1058.
19. Schroeder A, van der Zypen E, Stich H, Sutter F. The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. J Maxillofac Surg 1981;9:15–25.
20. Tan WC, Ong M, Han J, Mattheos N, *et al*., on Behalf of the ITI Antibiotic Study Group. Effect of systemic antibiotics on clinical and patient-reported outcomes of implant therapy- a multi-centre randomised controlled clinical trial. Clin Oral Implants Res 2014;25: 185–193.
21. Wilson TG Jr, Schenk R, Buser D, Cochran D. Implants placed in immediate extraction sites: a report of histologic and histometric analyses of human biopsies. Int J Oral Maxillofac Implants 1998;13:333–341.
22. Wilson TG Jr, Carnio J, Schenk R, Cochran D. Immediate implants covered with connective tissue membranes: human biopsies. J Periodontol 2003;74: 402–409.