Experimental procedure for the evaluation of the mechanical properties of the bone surrounding dental implants

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Abstract

The mechanical stability of the fixture in bone is one of the most important factors for the long-term reliability of dental implants. This paper focuses on an experimental procedure to evaluate the mechanical properties of the bone surrounding dental implants. The procedure is based on a surgical animal model followed by mechanical tests. The experimental mechanical testing has been used for preliminary investigations on the role played by different parameters such as the healing time and the surgical technique (standard or with regenerative material). The procedure has been evaluated in some preliminary tests on a few specimens. Microradiographic analyses have been performed on the bone surrounding the implants in order to give an interpretation of the bone properties on the basis of the bone morphology and to distinguish the newly formed bone from the pre-existing bone. The preliminary results relevant to 10 threaded titanium implants are presented and discussed. Our findings show that the mechanical properties of the bone surrounding the implant improve with the increase in the healing time from 24 to 45 days. The ultimate loads recorded during mechanical tests arise from 395 N to 2665 N in case of coronal defects filled with bone regenerative and from 2200 N to 5700 N in case of standard technique.

Keywords: Mechanical test; Bone mechanical properties; Guided bone regeneration (GBR)

1. Introduction

The long-term reliability of prosthetic rehabilitation supported by dental implants depends on several factors. The surgical technique, the pre-operative bone properties [1], the fixture osseointegration during the post-operative period without loading and the load distribution during chewing [2–5] are the most important elements that influence the prosthetic survival. The most frequent cause of implant failure is the loosening of the bone–fixture interface and the micromovements between the two, which leads to implant removal. Looking at the bone as a fixed constraint for the fixture, two subsequent phases should be considered in evaluating the bone–fixture interface. In the first phase, the post-operative stability is usually obtained using a proper surgical technique and proper implant hardware. In the second, the long-term stability depends on the bone adaptation to the stress pattern induced by the fixture. It follows that for the proper evaluation of the long-term stability of the fixture it is fundamental to take into consideration the mechanical properties of the bone surrounding the implant as a remodelling tissue. It is well-known that the morphology of a bone is first established by genetic factors and afterward the bone goes through dynamic shape and density optimisation to adapt its mechanical properties and structural behaviour to the local stress [6]. As a consequence, it follows that the bone surrounding the fixture represents a dynamic constraint, showing evolution in time according to the response of the tissue to the stress induced by the fixture supporting the dental prosthesis. Soon after the fixture

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insertion, the problem concerns the creation of the interface, whose stability depends upon the absence of relative motion between fixture and bone and the capability of bone cells to establish osseointegration. After the anchorage of the fixture has been achieved, the interface stability may be lost not only under high peaks of load which may produce bone microfractures, but even for the local resorption of bone due to the functional adaptation to the stress pattern [7,8].

Furthermore, the bone–titanium interface strength depends on the pre-operative bone quality and hence the pre-operative bone mechanical properties [1,8–13].

The guided bone regeneration (GBR) technique, aiding the bone formation, allows the insertion of the fixtures even if the bone thickness and height are poor or in post-extractive sites. The histology of the regenerated bone shows the reliability of the GBR techniques [14–16], but longer bone healing time could be needed before loading the fixtures [17]. Despite the extensive use in clinical practice of GBR techniques, no definitive results are available yet concerning the mechanical properties of the bone surrounding the implant when regenerative bone substitutes are used to fill bone defects [18].

Meredith and co-workers [19–21] have carried out several studies to assess the stability of the fixture–tissue interface giving a quantitative indirect determination by means of resonance frequency analysis. Johanson and co-workers [22–26] have evaluated the torque required to remove titanium implants fixed in tibia and femur diaphyses and metaphyses of animals, while Tjellström et al. [27] made a similar study using mastoid human cadaver bone and Ueda et al. [28] using both mastoid and temporal human cadaver bone. Carr et al. [29] have evaluated the torque failure on screw-shaped implants in baboon mandibles in order to estimate the dependence of torsional resistance values on biomaterials; implant position (maxilla or mandible) and healing time. Berzins et al. [30], Ivanoff et al. [31] and Kraut et al. [32] have focused their work on in vitro pull-out tests that allow the evaluation of the resistance of the bone–fixture interface.

The mechanical stability of the implant in the bone depends on both the osseointegration and the mechanical properties of the surrounding bone. From the clinical point of view, the mechanical stability of an implant into the bone requires that even the bone surrounding the implant should not fail or fracture under load. With torque tests it is possible to measure the osseointegration, while by means of push-in test we measure the capability of the bone tissue surrounding the fixtures to support the threaded implant, and hence the mechanical properties of the bone tissue as a constraint. The portion of bone surrounding the implants that we are going to evaluate is the bone tissue extending as far as 2 mm around the implant external surface.

This paper proposes a procedure for the evaluation of the mechanical properties of the bone surrounding the implants. The experimental procedure consists of push-in tests on a specimen obtained after the surgical application of threaded implants in sheep tibia. The procedure has been used for preliminary investigations on different surgical techniques and different healing times. In particular, the mechanical characteristics of natural bone regenerative activity with respect to the bone regeneration artificially driven by bone grafting and membranes at two different healing times have been evaluated. The microradiographic analysis of the bone surrounding the fixture has also been carried out to give the interpretation of the biomechanical findings.

2. Method

The experimental procedure consists of three steps: the surgery, the mechanical test and the microradiographic analysis. In the following, each of the three steps will be described along its main guidelines.

2.1. Surgical protocol

Surgery was required to prepare the specimens for the mechanical tests and consisted in the insertion of some fixtures in sheep tibia.

After having anaesthetised the animal, the tibia was exposed to allow the bone site preparation, by means of the surgical tools accompanying the implant system and refrigerating the burs with saline. The peri-implant coronal bone defects were created by means of a countersink. In this occurrence, the gap surrounding the fixture was filled with deantigenate bovine bone particles (Bio-Oss®, Geistlich, CH). A resorbable Polylactin membrane (Vycril® TM, Johnson & Johnson, USA) was applied to cover the surgical site. After the insertion of the fixture and the application of the healing cap, the skin was sutured to close the surgical site. Two osseous defects, one for each sheep, were created likewise, filled with Bio-Oss® and covered with Vycril® membrane, in order to be used as control sites. To localise the bone defects in the following stages, Memphix screws were inserted marginally to the lesions.

The animals were stabilised for the required time before the sacrifice. A bone specimen including the fixture, when present, was cut out after the removal of the soft tissues.

The specimens were stored in 4% paraformaldehyde until the mechanical testing.

2.2. Mechanical test procedure

The bone specimens were fixed in a cylindrical support using a glass-ionomer cement. During the
cement hardening the bone specimens were kept in place using the fixture axis as a reference. To guarantee the alignment between the fixture axis of the specimen and the load axis of the testing machine, an abutment was screwed to the fixture and it tip glued on the seat of the load applying device as shown in Fig. 1. A MTS 858 MiniBionix (MTS Minneapolis, USA) testing machine, equipped with a 10 kN load cell was used to apply the load. Force–displacement curves were recorded under displacement control with a displacement rate of 0.25 mm/min until the ultimate load has been achieved and the recorded load decreased. The ultimate load is the maximum load recorded during push-in tests.

The load applied to the implant during the mechanical test generates a shear stress in the bone tissue. The maximum stress develops on the external profile of the threads in the coronal part of the fixture, which corresponds to the portion of the surrounding bone that we have evaluated during the mechanical test as shown in Fig. 1. The samples have a square section of 10 mm size, which contain the breaking surface. The ultimate load values have been used to evaluate the mechanical properties of the bone surrounding the implants for comparison under different conditions of healing times and used techniques.

2.3. Microradiographic analysis

Beside each fixture a second fixture was inserted in sheep tibias to perform microradiographic analyses. Under the assumption that the two bone–fixture interfaces have the same characteristics and mechanical properties, we are allowed to analyse the microstructure of one and utilise it to give an interpretation of the mechanical behaviour shown by the other during the breaking test.

The bone segments containing the implants were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.2 for 6 h at room temperature, washed in buffer for 10 min, then dehydrated through 60%, 70%, 80%, 95% and two changes of absolute ethanol, 24 h each. Afterward the specimens were immersed for 24 h in a mixture of methylmethacrylate and absolute ethanol (50%), for 24 h in pure methylmethacrylate, for 48 h and three changes in the infiltrating solution: 100 g of methylmethacrylate, 15 g of Triton N101, nonylphenylether of polyethylene glycol and 3 g of benzoyl peroxide.

The bone specimens were then placed in a flat-bottomed polyethylene container fully filled with the polymerising solution, 15 g of PMMA in pearls dissolved in 100 ml of infiltrating solution, hermetically sealed, and left to polymerise for 36/72 h in a water bath at 37°C. A series of transverse sections (200 μm thick) were obtained using a 1600 Leica (Wetzlar, D) circular diamond microtome. The surfaces of each section, polished with emery paper and alumina, were microradiographed at 6 kV and 2 mA using an Italstructures device and high resolution Ilford EM film.

3. Materials

A total amount of 10 self-tapping titanium fixtures (Tioblast™ Fixture, Astra Tech, S), with a diameter of 3.5 mm and the surface blasted with titanium dioxide were placed in tibial proximal sites of two 18 months old sheep of 80 kg weight (5 fixtures for each sheep). The two sheep do not have any differences. Fig. 2 shows the anatomical drawing of the location where the fixtures were inserted. The fixtures were inserted along a curved line parallel to the tibial border to avoid the medullary channel and to get the best primary stability. The aim was to install the fixtures in the trabecular bone avoiding any distal contact with the cortical bone. The distance between the edges of the fixture was about 3 cm.

Six Tioblast™ fixtures were placed in surgically prepared 3.20 mm diameter sites with coronal peri-implant round defects (specimens identified as S1B, S1C, S1D, S2B, S2C and S2D). The defects were filled with Bio-Oss® and covered with a Vycril™ membrane. The other four Tioblast™ fixtures were inserted without creation of defects (specimen identified as S1A, S1E, S2A and S2E).

The two sheep were sacrificed with an intrapulmonary Tanax™ (Hoechst AG, Frankfurt, D) injection after 24 days (specimen identified as S1) and 45 days (specimen identified as S2) to evaluate the influence of the healing time.
Table 1 summarises the data for all the tested specimens. Six fixtures (S1B, S1C, S1A, S2B, S2C and S2A) were designated for the mechanical tests. The other four fixtures (S1D, S1E, S2D and S2E) and the two control specimens, one coming from the sheep sacrificed at 24 days (S1F) and the other from the sheep sacrificed at 45 days (S2F), were addressed to the microradiographic analysis.

4. Preliminary results

The ultimate loads measured for each specimen which had undergone the mechanical test are reported in Table 1.

Fig. 3 displays the force–displacement curves obtained for the sheep specimens. Fig. 3a shows the comparison between a bone specimen (S1A) with the fixture inserted using the standard technique and two specimens (S1B and S1C) with peri-implant surgical defects filled with bone substitute. The results show that the specimens with defects filled with Bio-Oss® have measured ultimate loads lower than the specimen without defects. This shows that after a healing time equal to 24 days the regenerated bone starting from Bio-Oss® does not achieve the same level of properties of the healthy bone surrounding the fixture after the standard technique. Fig. 3b shows the effects due to the bone healing time on the mechanical properties of the bone surrounding the fixture. The curves refer to specimens coming from the sheep sacrificed 24 (S1B and S1C) and 45 (S2B and S2C) days after surgery. This comparison shows that, in case of GBR technique, the healing time increasing from 24 to 45 days improves the mechanical properties of the bone and consequently, the stability of the fixture. The ultimate load measured for the specimens with surgical defects and 45 days of healing time (S2B and S2C) is comparable with the results obtained for the specimen S1A inserted using standard technique and with 24 days of healing time.

Microradiographic analyses have been performed to provide further information on bone microstructure and morphology. As regards the microradiographic analyses of specimens with surgical defect coming from the sheep sacrificed 24 days after surgery, specimen S1D appears to be surrounded by a great number of newly formed trabeculae (Fig. 4a). In particular, the apex is completely surrounded by the bone. In the grooves between the

Table 1
Summary of the specimen data

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Surgery type</th>
<th>Bone regenerative</th>
<th>Vycril membrane</th>
<th>Healing time (days)</th>
<th>Ultimate load (N)</th>
<th>Morphological analysis</th>
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<tr>
<td>S1A</td>
<td>No defect</td>
<td>No</td>
<td>No</td>
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<td>2200</td>
<td></td>
</tr>
<tr>
<td>S1B</td>
<td>Coronal defect</td>
<td>Bio-Oss®</td>
<td>Yes</td>
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<td>370</td>
<td></td>
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<tr>
<td>S1C</td>
<td>Coronal defect</td>
<td>Bio-Oss®</td>
<td>Yes</td>
<td>24</td>
<td>420</td>
<td></td>
</tr>
<tr>
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<td>Bio-Oss®</td>
<td>Yes</td>
<td>24</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>S1E</td>
<td>No defect</td>
<td>No</td>
<td>Yes</td>
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<td></td>
<td>Yes</td>
</tr>
<tr>
<td>S1F</td>
<td>No fixture</td>
<td>Bio-Oss®</td>
<td>Yes</td>
<td>24</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>S2A</td>
<td>No defect</td>
<td>No</td>
<td>No</td>
<td>45</td>
<td>5700</td>
<td></td>
</tr>
<tr>
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<td>45</td>
<td>2390</td>
<td></td>
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<tr>
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<td>Bio-Oss®</td>
<td>Yes</td>
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<td>2940</td>
<td></td>
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<tr>
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<td>Coronal defect</td>
<td>Bio-Oss®</td>
<td>Yes</td>
<td>45</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>S2E</td>
<td>No defect</td>
<td>No</td>
<td>Yes</td>
<td>45</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>S2F</td>
<td>No fixture</td>
<td>Bio-Oss®</td>
<td>Yes</td>
<td>45</td>
<td></td>
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</tr>
</tbody>
</table>
threads there are thin trabeculae in contact with the fixture. The compact pre-existing bone that was in contact with the apaxes of the threads at the moment of insertion does not appear to be involved in the osteoclastic erosion being still in contact with the titanium (Fig. 4b). The control hole (S1F) appears to be completely open; however, 24 days after surgery its diameter is reduced (Fig. 5). Thin newly formed bone trabeculae branch off both from the wall of the hole and from residuals of the pre-existing bone; the erosion that usually follows the surgical creation of a hole spared these.

Specimen S2D coming from sheep sacrificed after 45 days appears to be surrounded by newly formed bone (Fig. 6a). The titanium interface is in contact with the bone that accomplishes a satisfying osseointegration of the fixture. The newly formed bone starts from portions of pre-existing one. In the surgical defect, the newly formed bone comes from a thick and hypercalcified cementing line that separates it from the pre-existing bone. This line is in contact with the titanium (Fig. 6b). A few granules of Bio-Oss® appear to come into contact with the newly formed bone, whereas the greater part of the grains is wrapped by fibrous tissue. As regards the specimen without defect (S2E) it appears to be surrounded by newly formed bone (Fig. 7a). The fixture surface is in contact directly with the newly formed bone (Fig. 7b). In the control hole the newly formed bone reduces the diameter of the hole to one-half of the original width and appears to be made of thickened trabeculae, with small and long vascular spaces that have a radial direction (Fig. 8). The Bio-Oss® filled bone seems very similar to the one examined 24 days after insertion. However, the bone trabeculae, that are constructed starting from the compact plate endosteal surface and that internally close the defect, appear to be more thickened.

5. Discussion

The evaluation of the properties of the bone surrounding the fixtures is one of the most critical aspects for the success of a dental implant. Different approaches such as resonance frequency analysis [19–21], counter torque tests [22–29] and pull-out tests [30–32] have been used to measure its strength in order to give a quantitative evaluation of the achieved osseointegration.

We have presented an experimental procedure to evaluate the mechanical properties of the bone surrounding the fixture, consisting of push-in tests on specimens obtained after the surgical insertion of threaded implants in sheep tibia. This new method has been used for preliminary investigations on two different surgical techniques (standard technique and GBR technique) and two different healing times (24 and 45 days). Microradiographic analyses of the bone surrounding the fixture have also been carried out to give the interpretation of the biomechanical findings.

This study has been conducted in order to evaluate the mechanical properties of bone surrounding the fixture, as the capability of the bone tissue to be a mechanical constraint for the threaded implant. Hence, the ultimate load measured during the mechanical testing is related to the mechanical properties of the bone surrounding the fixture and it is not related to the osseointegration at the bone–fixture interface. Torque tests are usually adopted to evaluate the osseointegration achieved between the fixture and the surrounding bone. This kind of test allows the determination of the capability of the fixture surface or the capability of the applied surgical technique to drive the osseointegration in order to achieve the fixture anchorage.

The advantage of this experimental procedure is its capability to evaluate the mechanical properties of the bone surrounding the fixture, which represents the
mechanical constraint for the inserted implant, reproducing the same kind of loading that occurs during chewing or occlusion, when fixtures are mainly subjected to push-in loads. The main limitations of the presented technique concern the difficulty to achieve the alignment between the fixture axis of the specimen and the load axis and the presence of cortical bone under the fixture that increases the compressive strength.

The first aspect is taken into account in this experimental procedure by screwing an abutment to the fixture and gluing its tip on the seat of the load applying device. This method allows one to obtain the alignment of the fixture, containing the inclination error due to the manual positioning of the abutment on its seat within a range of a few degrees. The second aspect is more difficult to be controlled and impossible to be standardised, since the presence of cortical bone in

Fig. 4. Microradiographic analyses of specimen S1D with coronal defects coming from the sheep sacrificed at 24 days. The radiographic analyses show that the specimen appears to be surrounded by a great number of newly formed trabeculae in the coronal part (a1), in the middle part (a2) and in the apical part of the fixture (a3), while the compact pre-existing bone is still in contact with the titanium (b).

Fig. 5. Microradiographic analysis of the control hole (S1F) coming from the sheep sacrificed at 24 days.
contact with the fixture in the apical region depends on surgery and tibia anatomy. During surgery, attention was paid to the orientation of the fixture in bone in order to have a vertical insertion, avoiding the contact between some parts of the fixture and the cortical bone of the lower layer. With regard to the precise insertion of the fixture in the centre of the coronal defects filled with Bio-Oss \(^{1}\), there were no difficulties as the defect was created purposely by means of a countersink. Even if the surgery were conducted carefully, it would not be possible to guarantee that some edges of the implant were not anchored to the original host bone. This represents a potential limitation of the mechanical testing method.

We have verified the applicability of our mechanical testing method to investigate the effects on bone properties of both the surgical techniques and healing times. Even if our preliminary results on few specimens do not provide statistical analysis to support the conclusions, a brief discussion about the preliminary investigations conducted on different surgical techniques and different healing times follows.

On the basis of the results of the mechanical testing one can note that the healing time influences the

Fig. 6. Microradiographic analyses of specimen S2D with coronal defects coming from the sheep sacrificed at 45 days. The radiographic analyses show that the specimen is conspicuously surrounded by newly formed bone (a); and that the newly formed bone is constructed starting from a thick and hypercalcified cementing line in contact with the titanium (b). Indexes 1, 2 and 3 indicate the coronal part, the middle part and the apical part of the fixture, respectively.
The premature application of load to the implant during healing can destroy primary fixation causing the relative motion of the implant itself which may delay or prevent the osseointegration. The mechanical tests show that 45 days after surgery the fixtures inserted in regenerated bone can be subjected to the same loads supported by fixtures inserted by means of the standard surgical technique after 24 days, as the specimens with peri-implant coronal surgical defects, filled with bone substitute and coming from the sheep sacrificed 45 days after surgery (S2B and S2C), have ultimate load values comparable with the ultimate load recorded for the specimen S1A, installed without defects and coming from the sheep sacrificed 24 days after surgery.

Fig. 7. Microradiographic analysis of specimens S2E without defects coming from the sheep sacrificed at 45 days. The radiographic analysis shows that it is surrounded by newly formed bone (a). The newly formed bone is in contact with fixture surface (b). Indexes 1, 2 and 3 indicate the coronal part, the middle part and the apical part of the fixture, respectively.

Fig. 8. Microradiographic analysis of the control hole (S2F) coming from the sheep sacrificed at 45 days.
These results have been corroborated by the micro-
radiographic analyses that have given evidence that the Bio-Oss\textsuperscript{16}, as osseconductive material, favours bone-regeneration. In particular, the microradiographic analyses show that while after 24 days the neof ormation is still in its initial stage, after 45 days the control defects appear to be obliterated by about 50%. The presence of direct contacts between the newly formed trabeculae and all the portions of the implant surface—apex, grooves, threads, neck—are constant in the fixatures coming from the sheep sacrificed 45 days after surgery [33]. Both mechanical and microradiographic results confirm that GBR techniques may guarantee the bone regeneration and the interface stability when the bone maturation has been attained [17,18].

Further studies using animal bone specimens do not allow the extension of the results to mechanical properties of bone in humans, but allow a comparison of different techniques, materials and fixatures.

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References