

The use of a coin shaped implant for direct in situ measurement of attachment strength for osseointegrating biomaterial surfaces

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Abstract

Most animal models currently used to study the retention of implants in bone are influenced by shear forces introduced during the retention test. This is mainly due to the implant design, which most often are cylindrical, conical or threaded. In these models interlocking between bone and implant surface will increase the effect of genuine bone bonding and thus give a false positive outcome. The purpose of the present study was to establish a model for testing functional attachment of implants in situ, with minimal influence of interlocking and shear forces. The model involves the use of flat coin shaped implant placed onto the cortical bone of rabbit tibia without mechanical fixation to the bone. The implant is passively retained on the cortical bone by a titanium band retainer. During the healing period, the contact between the coin shaped implants and the bone is restricted to the flat test surfaces. To prevent interlocking effects from lateral bone attachments a polytetrafluoroethylene (PTFE) cap covering the vertical and the upper faces of the implants were used. The tensile test was performed with a gradual, calibrated pull, perpendicular to the bone-implant interface. This pullout model makes it possible to study the kinetics and strength of bone bonding with negligible influence of shear forces or mechanical interlocking. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Biomaterials; Attachment strength; Surface roughness; Tensile testing; Bone bonding; Perpendicular forces; Titanium implants; Animal model

1. Introduction

Titanium and Ti-alloys appears to be the material of choice for integrated load bearing implants in bone, but many factors influence the incorporation and function of such implants in the body. The surface roughness at micro and macro levels, as well as the nature of chemical groups exposed on the surface of the implants, has been demonstrated to influence the attachment [1–5]. Much interest has been concentrated on the surface roughness of the implant. Surface roughness may introduce several mechanically based effects in bone such as interlocking due to bone in-growth into the surface and influence of load distribution to the surrounding bone [6–8]. Rough surface structure may also stimulate growth and repair of the bony tissue. Implant surface improvements having effect on the bone-to-implant contact have gained considerable attention focus over the last years.

However it has proven difficult to separate biological effects from mechanical retention in commonly used test systems for implant attachments. Thus, it is important to develop test methods for such modified implant surfaces that can discriminate between different mechanisms for implant attachment.

Different animal models have been developed to gain information about attachment between tissue and implanted materials. Implant retention can be considered to be a net sum of three major parameters: friction, mechanical interlocking, and chemical bonding [9]. Friction and mechanical interlocking theoretically decrease with increasing taper, whereas this do not influence a genuine bonding between the bone and a biomaterial. Increasing surface roughness may lead to greater mechanical interlocking and may thus further enhance interface shear strength. Most studies on bone attachment of titanium implants have focused on the effect of shear forces using pushout [10–12], pullout [13–15] or torque tests [16–18]. Pushout and pullout tests however, have several limitations for evaluation of the

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attachment between bone and implant. The implant design i.e. the tapering, the degree of surface roughness, and the alignment of the plug related to the support jig, all influence on the test results [19].

Removal torque tests have the theoretical advantage of providing an equal force distribution field at the bone–implant interface. On the other hand, these experiments are more complicated and time consuming than pushout or pullout tests [20]. More important, torque tests still do not provide clear-cut information about bone bonding and attachment alone since observations are also strongly influenced by friction and in-growth of bone. Thus, torque tests do not necessarily discriminate between bone bonding and mechanical retention.

Some studies have focused on measurements of tensile strength [19,21–27], that according to some authors, only can be caused by chemical bonds between implant and bone [28,29]. Thus, it is believed that with a tensile test method, the strength of the chemical bonding can be measured directly, minimising the effects of friction and of mechanical forces introduced by surface roughness [19]. Steinemann and co-workers [21] addressed these aspects and presented a tensile testing model that minimises the influence of shear forces. Despite of this, this model has not frequently been utilised and other model systems dominate the literature.

The aim of the present study was to develop an animal model specially designed for testing bone bonding of

titanium implants. As in the previous described tensile testing models, the model presented here involves the use of coin shaped implants that is placed flat on a levelled bone surface. The model allows direct observations of the tissue-implant interactions that influence the strength of bone bonding without being masked by mechanically forces like friction or interlocking. This model could thus contribute to the further study of biological and biochemical mechanisms involved in adhesion of titanium implants to bone.

2. Materials and methods

2.1. Implants

Calibrated coin shaped implants with a diameter of 6.25 mm and a height of 1.95 mm, were made from 10 mm cylindrical bars of grade 2 titanium (ASTM B 348) (Fig. 1). On the reverse side a non-penetrating 1.5 mm deep, threaded hole was made for attachment to the testing machine (Lloyds LRX Materials testing machine). The test surface of the implants were standardised by polishing with a diamond abrasive polishing paste with 6 μm grain size, according to Struers[®] Metalog Guide. The test surfaces was then blasted with titanium dioxide (TiO_2) particles yielding an average S_a value of 1.43 and a S_{dr} value of 1.60, as measured in a confocal laser scanning microscope

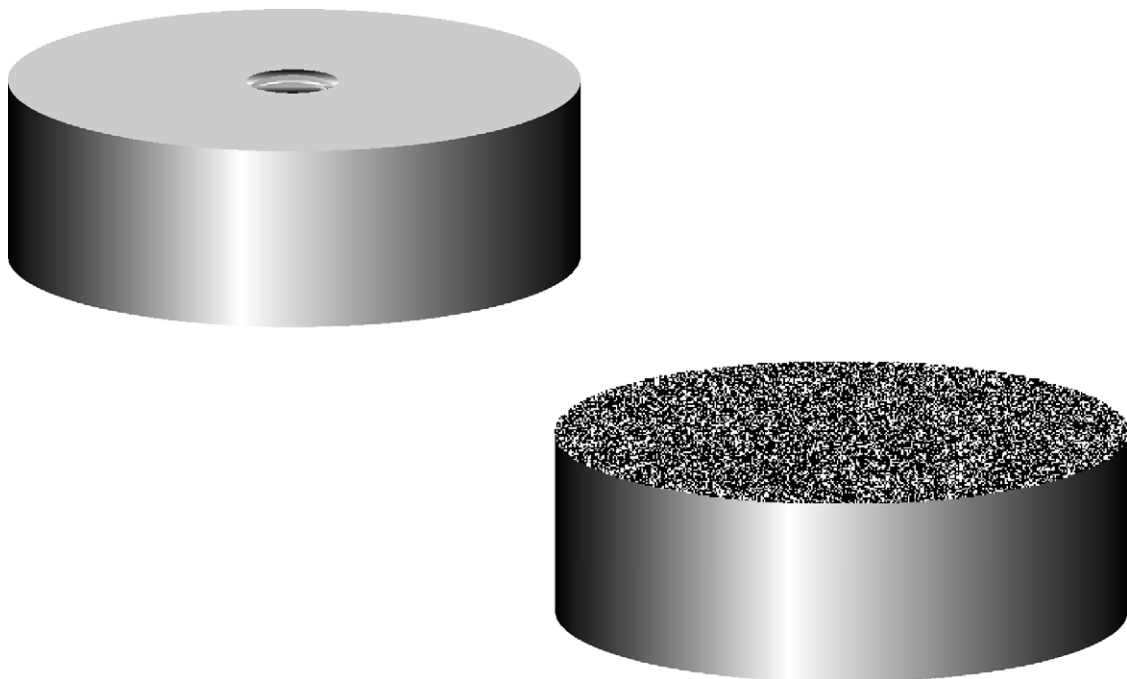


Fig. 1. The test implants used were all made of grade 2 titanium having a diameter of $6.25 \text{ mm} \pm 0.05 \text{ mm}$ and a height of $1.95 \text{ mm} \pm 0.01 \text{ mm}$. The test surface, which was placed in direct contact with the levelled bone, was blasted with TiO_2 particles with grain size $25 \mu\text{m}$. The vertical faces and the reverse side of the implant were not in contact with bone. The reverse side had a threaded hole in centre for attachment of the connector to the load cell.

(Leica, Germany). To ensure that the blasting did not change surface structure on the vertical faces of the implant, they were embedded into a 2 mm thick silicon rubber plate with 6.1 mm holes during the process.

Prior to surgery, all implants were cleaned with trichloroethylene followed by rinsing with 100% ethanol and subsequently by ethanol in an ultrasonic bath. A final rinse with deionised water at neutral pH was performed to ensure a clean surface. The implants were then autoclaved at 2 ATM, 137°C for 7 min.

2.2. Animals

A total of 12 New Zealand White adult female rabbits from Lidköping rabbit farm, Sweden, were used. The age was 8 months and the weight 3000–3500 g. The animals were housed in the Laboratory Animal Unit, The Norwegian School of Veterinary Science. During the experimental period the rabbits were held in cages. Room temperature was regulated to $19^{\circ}\text{C} \pm 1^{\circ}$ and a humidity of $55 \pm 10\%$. Fluorescent lightning in the cages was 20 lx in back 130 lx in front from 8.00 am to 8.00 pm. The air conditioning system changed air 15–20 times per hour. The animals were fed 160 g pelleted rabbit diet (B&K Universal AS, Norway) per day in addition to 15 g of autoclaved hay. Tap water, untreated, was available ad libitum from bottles. The microbiological status of the rabbits was examined according to a program by The Microbiological Laboratories, London, UK.

The experiments were conducted in accordance with the Animal Welfare Act of December 20th 1974, No. 73, chapter VI sections 20–22 and the Regulation on Animal Experimentation of January 15th 1996.

2.3. Sedation

The rabbits were sedated by injection with fluanisone/fentanyl (Hypnorm[®], Janssen, Belgium) 0.05–0.1 ml/kg s.c. 10 min prior to removal from the cages.

The animals were thereafter anaesthetised with midazolam (Dormicum[®], Roche, Switzerland) 2 mg/kg bw i.v. At sign of recovery during operation, diluted Hypnorm[®] (1 ml Hypnorm and 9 ml sterile water) was injected slowly i.v. until adequate effect was achieved, usually 0.1–0.2 ml at a time.

Lidocain/adrenalin (Xylocain/Adrenalin[®], Astra, Sweden) 1.8 ml s.p. was administrated locally at the operation site to achieve local anaesthesia and to control bleeding.

2.4. Surgery

Operation sites were depilated and washed with soft soap and ethanol directly before surgery, the operating sites were disinfected with 70% ethanol and the poste-

rior part of the animals was covered with a sterile blanket. An eye-ointment (Simplex[®], Nycomed Pharma, Norway) was used to protect the eyes from dryness during the procedure. A 5 cm incision was made on the proximal-anterior part of tibiae. The incision penetrated epidermis, dermis and the fascial layers. Lateral reflection of these tissues exposed the underlying periosteum. An additional medial-anterior incision was made through the periosteum. The periosteum was elevated with a periosteal elevator and retained by a self-retaining retractor (Fig. 2a). When the tibial bone of the rabbit is used, 4 implants can be placed in each animal, 2 in each leg. Four guide holes were made with a 1.0 mm diameter twist drill (Medicon[®], Germany) using a drill guide to ensure a standardised and correct positioning (Fig. 2b and c). With the use of a custom made 7.05 mm diameter stainless steel bur mounted in a slow-speed dental implant drill with copious physiological saline solution irrigation, a levelled platform was prepared for the test implants. (Fig. 2d and e). This was to ensure maximum contact between the test surface of the implant and the bone. The implants were stabilised by a pre-shaped 0.2 mm titanium maxillofacial plate (Medicon[®] CMS, Germany), retained in the cortical bone by two $1.2 \times 3 \text{ mm}^2$ titanium screws (Medicon[®] CMS, Germany) (Fig. 2f). This ensured a stable passive fixation of the implants during the healing period (Fig. 3). Polytetrafluoroethylene (PTFE) caps were introduced to resist bone growth towards the vertical faces of the implant as well as bone overgrowth (Fig. 4). The subcutaneous soft tissue was repositioned and sutured with 4-0 polyglycolic acid suture (Dexon[®] II, Sherwood-Davis and Geck, UK). The superficial layers were sutured back using an intra cutaneous technique with the same 4-0 suture. Each animal received an injection with 20 ml NaCl warmed to body temperature infusion s.c. and buprenorphin (Temgesic[®], Reckitt and Colman, England) 0.02–0.05 mg/kg s.c. following surgery. To relieve postoperative pain, a second injection of Temgesic, also 0.05 mg/kg s.c. was given at the end of the day—providing that at least 3 h had lapsed since the previous Temgesic injection.

The animals were then placed in their cages on an isolating plate with a bottle of hot water. The cages were covered with a cloth to prevent heat loss. The next morning, the rabbits received another Temgesic injection, this time at a dose of 0.035 mg/kg. Health condition was monitored during the study period and the operation sites were carefully examined daily until a complete wound healing.

2.5. Tensile test

The rabbits were euthanised by an i.v. injection of 1.0 ml fluanisone/fentanyl (Hypnorm[®], Janssen, Bel-

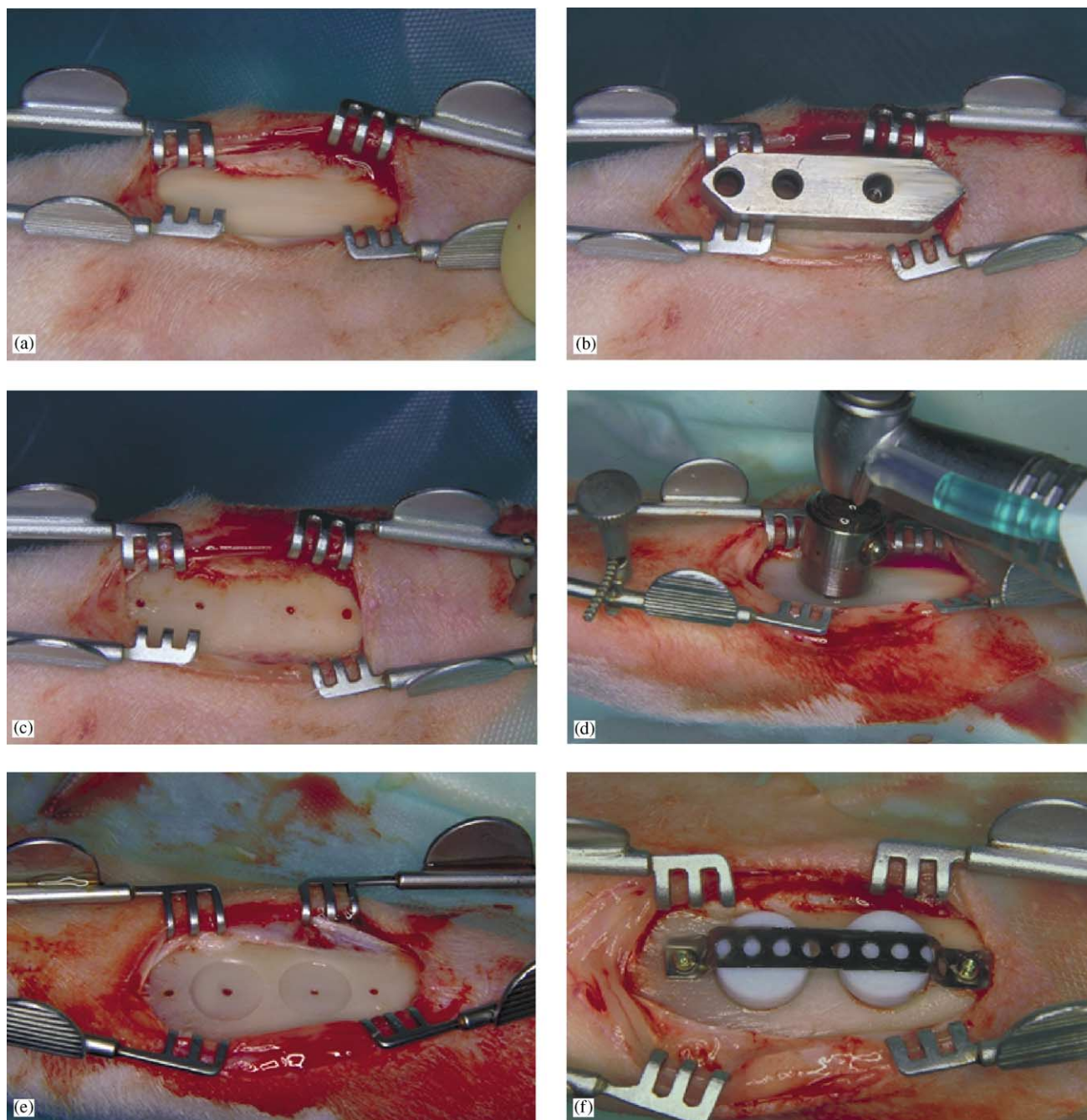


Fig. 2. The surgical procedure: (a) The exposed tibial bone. (b) The guide in place. (c) Tibial bone with guide holes. (d) Preparing the levelled site for the implants. (e) The levelled site for the implants. (f) The implants with PTFE caps placed and retained with the pre-formed titanium band.

gium) and 1 ml/kg bodyweight i.v. pentobarbital (Mebumal[®], Rikshospitalets Apotek, Norway).

Immediately after euthanasia the titanium plate covering the implants was exposed through an incision on the tibial bones. The implant fixations were then removed. A hole was made in the centre of the PTFE cap with a hypodermic needle, and air carefully injected through a syringe to separate the caps from the implants and expose the reverse part of

the implants. The tibial bone was then fixed in a special designed rig, to stabilise the bone during the tensile test procedure. A threaded pin with a ball-head was then attached to the implant by the use of the pre-made threaded hole and the set-up was adjusted perpendicularly to the load cell using a level tube. To minimise shear forces, a 300 mm long wire was connected between the load cell and the ball-head (Fig. 5).

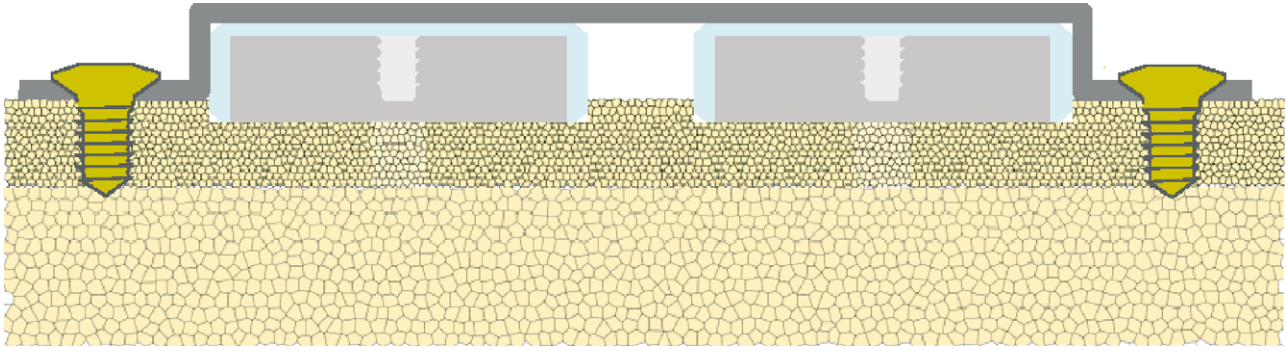


Fig. 3. Schematic drawing of a disc shaped titanium test implants covered with the PTFE cap, stabilised in the cortical bone with a titanium plate and retained by two titanium screws. The guide holes, for levelling implant sites, penetrated into the cancellous bone.

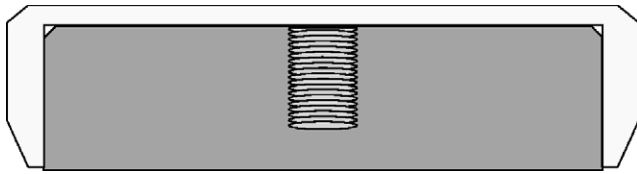


Fig. 4. A cross-sectioned, schematic drawing, of the test implant with a PTFE cap. Inside diameter of the cap is 0.02 mm less than implant diameter to insure good fit. In the centre of implants is a non-perforated threaded hole. The inside height of the cap was 0.05 mm less than the vertical height of the implant to ensure proper contact between implant and bone.

The tensile test was performed with a Lloyds LRX Materials testing machine fitted with a calibrated load cell of 100 N. Cross-head speed range was set to 1.0 mm/min. Force measuring accuracy was $\pm 1\%$ (Certificate of calibration: NAMAS Calibration No. 0019 Issued by Instron Calibration Laboratory no. 1000356). The load was applied until the implant detached from the bone and the force applied was recorded on a load versus displacement plot (Fig. 6).

3. Results

3.1. Material characterisation

Scanning electron microscopy (SEM) analyses of the test surfaces of the implants were performed before placing and after pullout to display surface structure details.

Pre-implantation quantitative data from a Leica TCS 4D confocal laser microscope yielded a mean deflection value of (S_a) of $1.43 \mu\text{m}$ and a maximum peak-to-valley roughness (S_t) of $31.76 \mu\text{m}$ and a developed surface area ratio (S_{dr}) of 1.60. Area measured was $1000 \times 1000 \mu\text{m}^2$.

3.2. Experimental

The retention between the implant and bone was evaluated immediately following euthanasia before

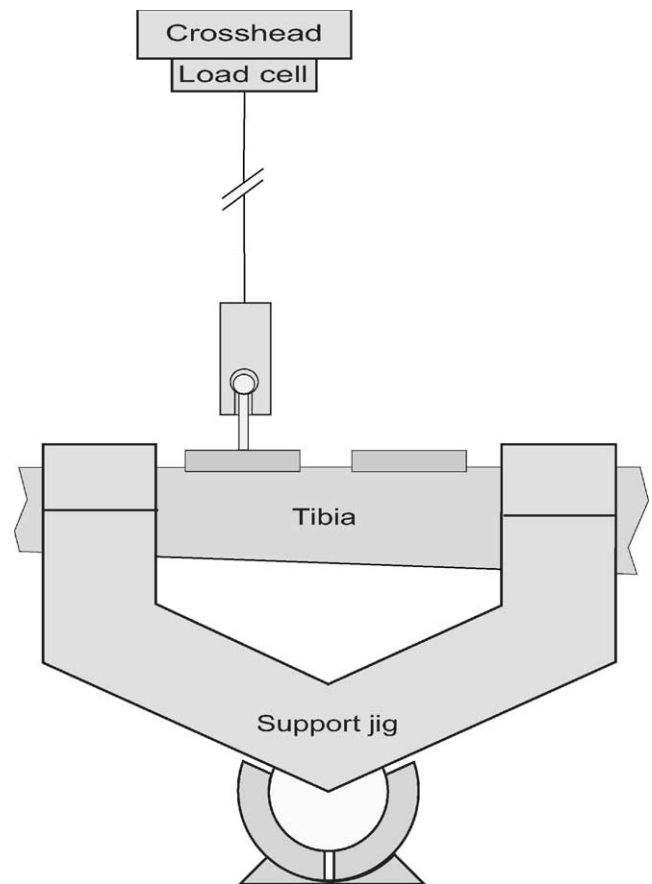


Fig. 5. Tensile test set-up. The tibial bone is fixed in the rig and the implant attached to the load cell by a threaded pin and a 300 mm long wire. The testing machine was a Lloyd LXR with a calibrated load cell of 100 N with an accuracy of $\pm 1\%$. Cross-head speed range was set to 1.0 mm/min.

drying could influence the results. As the load versus displacement was recorded (Fig. 6), it was possible to analyse if shear forces skewed the test. An immediate drop in the curve would indicate little influence of shear forces whereas a tapering curve would indicate more presence of these forces. The test implants were detached from the underlying bone at a mean force of 3.260 N

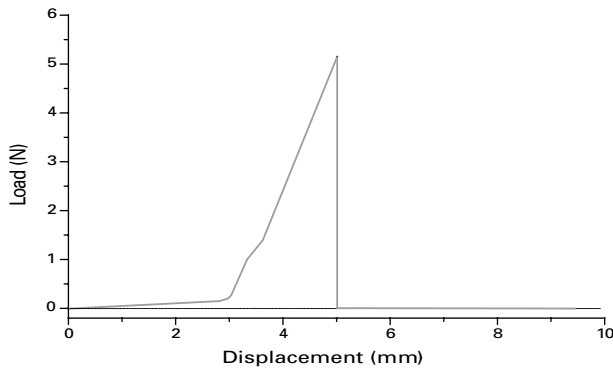


Fig. 6. Example of a load-displacement curve registered during a tensile test. The well-defined breakpoint and vertical drop in load indicates a sudden detachment of the test implant.

with a standard deviation of 1.521. Out of 48 implants, 18 were lost during the procedure when removing the cap, titanium band or when attaching the pin. These implants were not included in the experimental data. When calculating stress following the tensile test, the implant area was measured as projected and no consideration was taken to the measured developed surface area ratio (S_{dr}). Based on this estimate, the mean stress of the 30 implants was 0.110 MPa, standard deviation 0.0496.

The coefficient of variance (CV) of the tested implants was 47%. The mean values were strongly affected by the outlier. The CV was reduced to 33%, with elimination of one outlier (Fig. 7).

4. Discussion

The biological effect and clinical outcome of titanium implants in bone can currently best be evaluated by the use of animal studies. Several different animal models are used to assess important success parameters for this purpose. Results from these models are often used as evidence for commercial claims for bone-binding capacity or as a merit of an implant design [19,21,24–26,30]. However most methods used today rarely mimic real clinical situations nor do they provide specific information on specific retention and attachment mechanisms.

In most pullout and pushout tests unthreaded implants are used [10,11,13,15,31]. When increasing loads are applied onto these implants, the retention at the vertical faces of the implant in contact with bone, affect the measurements significantly [11]. An obvious important aspect of the performance of this technique is the complete removal of obstructive tissue that physically blocks movement of the implant. Dhert et al. [9] defined a number of test conditions crucial in pullout tests: The distance between the implant and the support

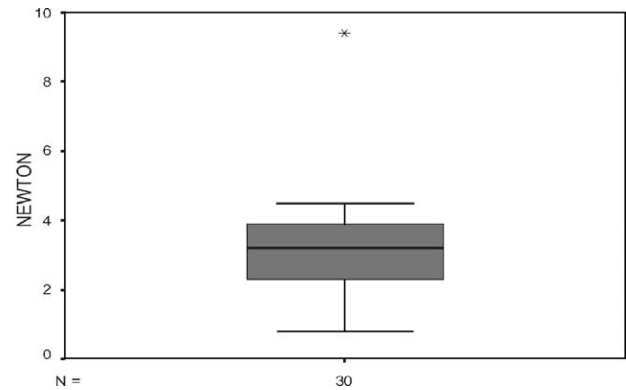


Fig. 7. Box plot of pooled data from the tensile testing of the four different test series. The forces applied to detach similar microrough implants ($N = 30$) in four different test series did not differ significantly indicating that results from different series can be compared with this standard test procedures. The CV was 47%.

jig was supposed to be critical to obtain a peak stress in the interface. Variations of the elastic modulus of the implants could result in a wide range of interfacial shear stresses. On the other hand, Dhert stated that variation of the cortical thickness and the diameter of the implant does not appear to have much influence on the interface shear stress [32]. However, the measured retention forces will to a certain extent be shear forces that are highly influenced by irregularities on the implant surface. This makes it difficult to discriminate between true bone bonding and mechanically interlocking, as described above. The nature of surface irregularities including the size, depth and width, will thus strongly influence the results of such studies. The bone quality at the implant site is also important since this is reflected in the strength of the bone growing into the irregularities. As a consequence of this, an implant placed into bone with a thick cortical lamella will presumably have a higher retention when measured with pullout or pushout tests, compared with an implant in bone with a thin cortical lamella. Increased surface areas also improve retention of the implant, especially when there exists biochemical binding between bone and the biomaterial [9]. Thus, in cases with high implant roughness and thick cortical bone, the retentive effect of the roughness can mask the actual bonding effect. Threaded implants have also been tested in pullout/pushout systems, but are considered to be unsuitable for this purpose due to the inherent high retention of such designs [9,33]. Threaded implants are more successfully evaluated by the use of removal torque tests [16–18]. By the use of such tests, the retentive effects of the implant surface can be measured. However, interlocking effects will influence the results also in this test method, due to bone in-growth into pores or other irregularities on the implant surface [14]. A flat or polished surface, as in the present study, needs a true binding to give resistance to both shear and

tension forces. In-growth of bone on rough surfaces may be sufficient to resist shear forces even in cases without a direct contact between bone and implant. However, according to Steinemann [21] rough surfaces do not resist tensile forces.

The present model was designed to reduce the problems of interlocking discussed above, and includes the advantages observed in previous coin-based models [21,26,27]. Due to the flat, “coin shaped” design of the implants, the influences of shear forces and the interlocking effects are minimised. The design is further improved by the use of PTFE caps that cover the coins, and thereby prevents bone growth onto the vertical faces of the implants. However, irregular porosities in the implant surface and subsequent bone growth into these, can probably also produce interlocking effects, in this model. This will, presumably, have less influence on the test results in the present model, than in pushout, pullout or removal torque test systems (Fig. 8). Many of the models used to test bone-to-implant reactions show a wide range of test conditions between individual studies and make comparisons difficult [9,20]. In particular the experimental designs, breed of animals, gender, age, the surgical techniques, choice of implantation sites, implant fixation, testing procedures and the cross-head speed, differ among individual experiments. A standardisation of the surgical technique, as well as drilling equipment and the precise placement of the implants, is necessary before results from different studies can be compared. The proximal tibial bone of rabbits is a good choice for a standardised model because the operation site in this area is relatively flat and large enough to allow the use of the flat, coin shaped implants.

In the present model the implants are not fixed to the bone, as is the case with threaded implants, but is rather stabilised by a pre-formed titanium band anchored to the bone with separate screws. A retention between the implant and bone after the healing period, will thus

solely depend on a reaction between the bone and the test surface of the implant, and thus not involve shear forces, undercuts or other mechanically based forces, making an assessment of true bone bonding more possible and reliable/reproducible.

The time from euthanasia to the performance of the test should be as short as possible. In some studies bone is stored in formaldehyde before the retention test is performed [34,35]. This is unfortunate because the retention of such implants may be twice as high as in implants processed within 4 h [36], probably because of tissue shrinkage caused by fixation by formaldehyde and subsequent removal of tissue water that can induce HAP crystallisation at the bone–implant interface. In the present model the time from euthanasia to the finishing of the tensile-test averaged 13 min. It should be emphasised that this tensile test could be performed in live, sedated animals, thus giving information on bone-to-implant bonding in living bone.

One of the problems with tensile testing, is the performance of the technically demanding pullout procedure [24]. This requires careful perpendicular alignment between implant surface and force direction. In the present model, this problem was minimised by the use of a 25 mm pin with a ball-head connected to a 300 mm wire as the connection between the horizontally aligned test implant and the vertically directed load cell (Fig. 4.).

After the tensile test is completed, it is possible to investigate the implant site with histological methods, since the bone is left intact after the tensile test procedure. In cases with very high bone–implant bonding, parts of the cortical bone may fracture and stick to the implant surface, but even this bone may be further investigated, by SEM or by chemical analysis. Quantitative and qualitative analyses of the test surface of the extracted implants may also give valuable information on the nature of the biological reactions involved in bone bonding and mineralisation.

The method described here for testing of bone–implant retention is standardised in certain major steps and described in details, making it possible to reliably reproduce the studies in other laboratories and thus enabling comparison of results from different series and even from different studies. The reduced influence of occluding forces, the lack of bone in contact with vertical faces of the implants, and the use of a standardised procedure may thus improve the quality and reliability of information concerning bone bonding mechanisms for titanium implants. If properly applied, this standardised model could benefit the investigating and development of optimal surface chemistry and structure for osseointegrating titanium implants and bring further scientific development in the field of osseointegrating implantology and medical materials science.

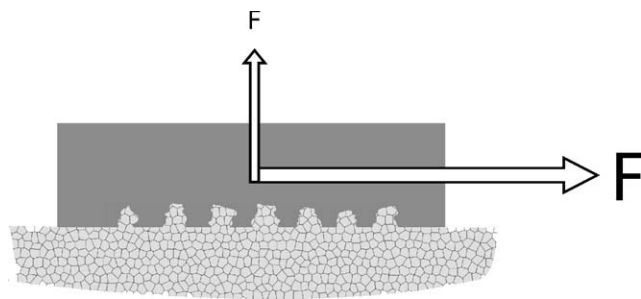


Fig. 8. Mechanical interlocking increases with decreasing angles between the load force direction and the implant surface. When the load force is perpendicular to the surface, retention by interlocking is nearly eliminated. Effect of interlocking due to micropores or irregularities in the implant surface will also be reduced in the presented model.

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References

- [1] Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand* 1981;52:155–70.
- [2] Taborelli M, Jobin M, Francois P, Vaudaux P, Tonetti M, Szmukler-Moncler S, Simpson JP, Descouts P. Influence of surface treatments developed for oral implants on the physical and biological properties of titanium. (I) Surface characterization. *Clin Oral Implants Res* 1997;8:208–16.
- [3] Ellingsen JE. Surface configurations of dental implants. *Periodontol* 2000 1998;17:36–46.
- [4] Ellingsen JE. Pre-treatment of titanium implants with fluoride improves their retention in bone. *J Mater Sci: Mater Med* 1995;6:749–53.
- [5] Ellingsen JE, Pinholt EM. Pretreatment of titanium implants with lanthanum ions alters the bone reactions. *J Mater Sci: Mater Med* 1995;6:125–9.
- [6] Wilke HJ, Claes L, Steinemann S. The influence of various titanium surfaces on the interface shear strength between implants and bone. In: Heimke G, Soltész U, Lee AJC, editors. *Clinical implant materials, advances in biomaterials*, vol. 9. Amsterdam: Elsevier, 1990. p. 309–14.
- [7] Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs [see comments]. *J Biomed Mater Res* 1991;25:889–902.
- [8] Wennerberg A, Albrektsson T, Andersson B, Krol JJ. A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. *Clin Oral Implants Res* 1995;6:24–30.
- [9] Dhert WJA, Jansen JA. The validity of a single pushout test. In: An YH, Draughn RA, editors. *Mechanical testing of bone and the bone-implant interface*. Boca Raton, FL: CRC Press LCC, 1999. p. 477–88.
- [10] Nakashima Y, Hayashi K, Inadome T, Uenoyama K, Hara T, Kanemaru T, Sugioka Y, Noda I. Hydroxyapatite-coating on titanium arc sprayed titanium implants. *J Biomed Mater Res* 1997;35:287–98.
- [11] Vercaigne S, Wolke JG, Naert I, Jansen JA. Histomorphometrical and mechanical evaluation of titanium plasma-spray-coated implants placed in the cortical bone of goats. *J Biomed Mater Res* 1998;41:41–8.
- [12] Ogiso M, Yamamura M, Kuo PT, Borgese D, Matsumoto T. Comparative push-out test of dense HA implants and HA-coated implants: findings in a canine study. *J Biomed Mater Res* 1998;39:364–72.
- [13] Berzins A, Shah B, Weinans H, Sumner DR. Nondestructive measurements of implant-bone interface shear modulus and effects of implant geometry in pull-out tests. *J Biomed Mater Res* 1997;34:337–40.
- [14] Branemark R, Ohnrell LO, Nilsson P, Thomsen P. Biomechanical characterization of osseointegration during healing: an experimental in vivo study in the rat. *Biomaterials* 1997;18:969–78.
- [15] Jinno T, Goldberg VM, Davy D, Stevenson S. Osseointegration of surface-blasted implants made of titanium alloy and cobalt-chromium alloy in a rabbit intramedullary model. *J Biomed Mater Res* 1998;42:20–9.
- [16] Wennerberg A, Albrektsson T, Lausmaa J. Torque and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-microns-sized particles of Al₂O₃. *J Biomed Mater Res* 1996;30:251–60.
- [17] Pebe P, Barbot R, Trinidad J, Pesquera A, Lucente J, Nishimura R, Nasr H. Countertorque testing and histomorphometric analysis of various implant surfaces in canines: a pilot study. *Implant Dent* 1997;6:259–65.
- [18] Branemark R, Ohnrell LO, Skalak R, Carlsson L, Branemark PI. Biomechanical characterization of osseointegration: an experimental in vivo investigation in the beagle dog. *J Orthop Res* 1998;16:61–9.
- [19] Nakamura T, Yamamuro T, Higashi S, Kokubo T, Ito S. A new glass-ceramic for bone replacement: evaluation of its bonding to bone tissue. *J Biomed Mater Res* 1985;19:685–98.
- [20] Berzins A, Sumner DR. Implant pushout and pullout tests. In: An YH, Draughn RA, editors. *Mechanical testing of bone and the bone-implant interface*. Boca Raton, FL: CRC Press LCC, 1999. p. 463–76.
- [21] Steinemann SG, Eulenberger J, Maeusli PA, Schroder A. Adhesion of bone to titanium. In: Christel P, Maunier A, Lee AJC, editors. *Biological and biomechanical performance of biomaterials*, vol. 6. Amsterdam: Elsevier, 1986. p. 409–14.
- [22] Yoshii S, Kakutani Y, Yamamuro T, Nakamura T, Kitsugi T, Oka M, Kokubo T, Takagi M. Strength of bonding between A–W glass-ceramic and the surface of bone cortex. *J Biomed Mater Res* 1988;22:327–38.
- [23] Lin H, Xu H, de Groot K. Tensile strength of the interface between hydroxyapatite and bone. *J Biomed Mater Res* 1992;26:7–18.
- [24] Kangasniemi IM, Verheyen CC, van der Velde EA, de Groot K. In vivo tensile testing of fluorapatite and hydroxylapatite plasma-sprayed coatings. *J Biomed Mater Res* 1994;28:563–72.
- [25] Takatsuka K, Yamamuro T, Nakamura T, Kokubo T. Bone-bonding behavior of titanium alloy evaluated mechanically with detaching failure load. *J Biomed Mater Res* 1995;29:157–63.
- [26] Edwards JT, Brunski JB, Higuchi HW. Mechanical and morphologic investigation of the tensile strength of a bone-hydroxyapatite interface. *J Biomed Mater Res* 1997;36:454–68.
- [27] Skripitz R, Aspenberg P. Tensile bond between bone and titanium: a reappraisal of osseointegration. *Acta Orthop Scand* 1998;69:315–9.
- [28] Gross U, Brandes J, Strunz V, Bab I, Sela J. The ultrastructure of the interface between a glass ceramic and bone. *J Biomed Mater Res* 1981;15:291–305.
- [29] Osborn JF. Bonding osteogenesis under loaded conditions—the histological evaluation of a human autopsy specimen of a hydroxyapatite ceramic coated stem of a titanium hip prosthesis. In: Oonishi H, Aoki H, Sawai K, editors. *Bioceramics*, vol. 1. Tokyo: Ishiyaku EuroAmerica Inc., 1989. p. 388–400.
- [30] Lin H, Xu H, Zhang X, de Groot K. Tensile tests of interface between bone and plasma-sprayed HA coating-titanium implant. *J Biomed Mater Res* 1998;43:113–22.

- [31] Lewis CG, Jones LC, Hungerford DS. Effects of grafting on porous metal ingrowth. A canine model. *J Arthroplasty* 1997;12:451–60.
- [32] Dhert WJ, Verheyen CC, Braak LH, de Wijn JR, Klein CP, de Groot K, Rozing PM. A finite element analysis of the push-out test: influence of test conditions. *J Biomed Mater Res* 1992;26:119–30.
- [33] Thomas KA, Kay JF, Cook SD, Jarcho M. The effect of surface macrotexture and hydroxylapatite coating on the mechanical strengths and histologic profiles of titanium implant materials. *J Biomed Mater Res* 1987;21:1395–414.
- [34] de Groot K, Geesink R, Klein CP, Serekian P. Plasma sprayed coatings of hydroxylapatite. *J Biomed Mater Res* 1987;21:1375–81.
- [35] Geesink RG, de Groot K, Klein CP. Chemical implant fixation using hydroxyl-apatite coatings. The development of a human total hip prosthesis for chemical fixation to bone using hydroxyl-apatite coatings on titanium substrates. *Clin Orthop Relat Res* 1987;225:147–70.
- [36] de Groot K. Letter to the editor. *J Biomed Mater Res* 1989;23:1367–9.