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## Method Article

# Considerations on the animal model and the biomechanical test arrangements for assessing the osseous integration of orthopedic and dental implants



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## A B S T R A C T

In implant research, a central objective is to optimize the osseous integration of implants according to their function and scope of application. In the preclinical stage, the animal model is commonly used to study implants for in vivo host tissue response and biomechanical tests are a frequently applied method for characterization of contact phenomena. However, the individual parameters and options for both the animal model and the biomechanical test arrangements vary widely, which can negatively affect the reliability and comparability of the results. In the present method description, we focus on implants for trabecular bone replacement and outline differentiated considerations for optimizing the animal model and the biomechanical test arrangement best suited for the area of application described. In addition, our aim was to present an optimized and strict study protocol for biomechanical push-out tests and step-by-step instructions in order to achieve precise and comparable results.

- The rabbit model and the distal femur as an implantation site are ideal for biomechanical assessment of implant osseointegration.
- Push-out tests are recommended, in which conformity of the axis is mandatory.
- Sequential examination periods are beneficial, e.g. after 4 weeks for osseoh healing and after 12 weeks for osseoremodeling.

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## Specifications table

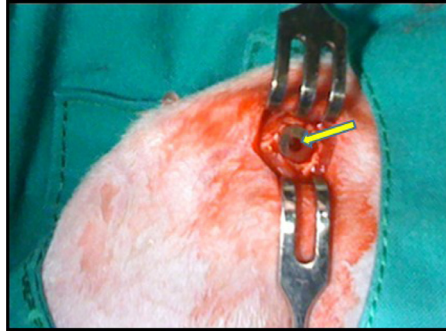
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Resource availability:	<i>If applicable, include links to resources necessary to reproduce the method (e.g. data, software, hardware, reagent)</i>

## Background

Cementless orthopedic and oral implants have been designed to increase osseous stability through a firm bond to the host bone, depending on their function and scope of application [32]. This is achieved either by ingrowth of bone in porous implants or by ongrowth (bone apposition) in the case of solid implants with a roughened surfaces. Osseointegration is particularly important with implants that rely on more biological anchoring in the host, such as graft replacements (e.g. trabecular bone defects, replacement of the intervertebral disc and fusion of vertebrae) than with implants that rely on a more mechanical anchoring (e.g. joint replacements, fracture fixating plates, dental implants). The term osseointegration originally marked by Branemark in 1976, which was used in dentistry for titanium implants (cp titanium) and their attachment to bones at the light microscopic level, has been expanded over the years to a more general definition including the osseous fixation of various kinds of orthopedic and dental implants by bone in/ongrowth due to a foreign body reaction [1,7].

The animal model is commonly used to study implants for *in vivo* host tissue response [32]. The selection of the species as well as other species-dependent model configurations such as the implantation site are strongly based on the nature of research question. Biomechanical push-out and pull-out test are frequently used methods for characterizing contact phenomena as an indication of the degree of osseointegration. However, the individual parameters and test arrangements vary widely, which can impair the results and make comparison with other studies more difficult [3]. Uniform biomechanical tests can hardly be achieved due to the different biomechanical test arrangements, material composition, shape and size of the implants and other varying individual parameters. These variations of animal model and biomechanical arrangements might alternate the results considerably and make a direct comparison difficult. In order to keep these deviations as low as possible, certain conditions and principles for the animal model and test arrangements should be adhered to as far as possible in order to improve the measurement accuracy and make the results more comparable. We previously submitted titanium alloy implants to a comparative biomechanical push-out study using an animal model to investigate the influence of different structure properties on osseointegration ([15]).

In this work, we present our considerations on the animal model used and on our strict, reproducible biomechanical test arrangement with step-by-step instructions based on the on the previous biomechanical study.



**Fig. 1.** Surgical site, lateral condyle of the rabbit femora. Sharp hooks hold the surgical wound open, the titanium cylinder has already been inserted press-fit, flush with the outer cortex. The central 2 mm drill channel follows the longitudinal axis of the implant (arrow).

## Animal model

### *Selection of animal model*

Rabbits are commonly used in animal models and show a number of advantages compared to other species, particularly when assessing the osseointegration of implants. They are ideal for use because of similar bone structure and fracture healing processes compared to humans, with primary bone healing being twice as fast [6]. The rabbit model offers a reliable and repeatable examination and assessment of osseointegration of implants that can be transferred to humans [34]. In addition, the rabbit bone size allows biomechanical and histological comparison with implants used in humans and is more independent of handling irregularities compared to smaller animals such as rats or mice. The rabbit animal model allows even from a veterinary perspective an animal species-rich husbandry with manageable effort, which would be more difficult with larger animal species such as pigs or dogs. We used female rabbits (Chinchilla Bastard) with an age of 12 to 14 weeks and a body weight not under 3 kg because their growth plates are almost closed and further growth disorders are not to be expected [21,24].

### *Implantation site*

The ideal implantation site for implants in the presented rabbit model certainly depends on the intended implant design and function as well as scope of application in humans. For use as a trabecular bone substitute, we have made the following considerations for implant positioning ([15]): (a) transchondral placement with probable gap formation may result in penetration of lubricant from the joint space into the interface [3]; (b) intramedullary placement or intra diaphyseal (axial) result in a large contact area with the endosteum and marrow; this probably fails to establish complete surface contact to the bone due to the conical shape mismatch leading to erroneously lower data [3,10,26]; (c) exclusive intracortical contact neglects on-growth of cancellous bone [4,5,22,33]; (d) intracondylar placement, as presented in this study, provides cortical and cancellous contact and strains applied to the cylinders [29,30].

The implantation site of the rabbit's lateral femoral condyle offers sufficient size and accessibility for the test implants used. (Figs. 1,2). In addition, the lateral condyle enables the examination of the endosteal cortical as well as the metaphyseal trabecular bone, which increases the relevance for clinical use in comparison to the sole diaphyseal placement [31]. The distal femur allows a constant load transfer during hopping, jumping and raising of the rabbit and a clinically relevant replication of human load distribution from the bone to the implant interface [31,32]. The bilateral implantation halves the number of used animals and enables a reliable and randomized comparison of implants with different properties ([14]) (Fig. 2).



**Fig. 2.** AP-radiographs of the harvested right and left femur of the rabbit. Titanium implants (white squares) in each lateral condyle 4 weeks after implantation.

### *The residence time of the implants*

The residence time of the implants was selected according to the physiological bone healing of the rabbit. After 4 weeks, we expect a partially excessive and disordered new bone formation from predominantly woven bone and larger portions of non-mineralized osteoid as part of the osseohaling [16,18,20]. After 12 weeks, the mid-term osseoremodeling, which represents an adaptation of the bone formation to the load direction, is predominantly developed. Histologically, we now expect mostly lamellar bones and a reduced presence of osteoid as well as a stronger bony anchoring ([8]; [15,34]). As a result, disorders of short-term osseohaling and mid-term osseoremodeling can be identified separately [5,25].

### **Biomechanical test arrangement**

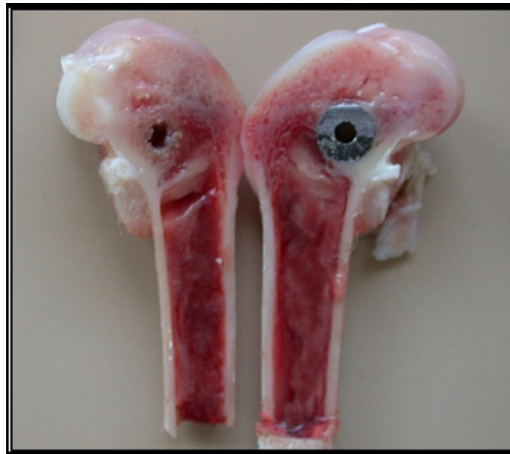
#### *Considerations for biomechanical tests*

#### *Selection of push-out criteria*

The shear strength as well as the push-out force are frequently used parameters for evaluating the bony stability of implants. Deglurkar et al. and Odgaard et al. discussed, however, that the positioning of the implant in both cortical and trabecular bone and varying surface structures of the implants creates an inhomogeneity of the interfaces between bone and implant, which calls into question the calculation of shear stress [3,10,27]. De Groot put the situation as follows: "It is better, in the meantime, to publish the push-out force, the failure mode, and the geometry of the implant,



**Fig. 3.** Exemplary porous (left) and solid, sand-blasted (right) Ti-6Al-4 V implants with a height of 7.0 mm and a diameter of 5.6 mm. The push-out device (middle) is 1.0 mm undersized in diameter compared to the implants to avoid contact with the bone during the push-out process. A central mandrel (arrow) of the device fits snugly into the central drill chanal of the implants.



**Fig. 4.** Trimmed distal femur with integrated implant 12 weeks after implantation. The exact alignment of the specimens in a sawing jig allowed a cut perpendicular to the longitudinal axis of the implant, as can be seen from the plane-parallel cuts along the outer circular plane of the implant (right).

rather than only give the quotient of force and some arbitrary area." [9]. Accordingly, we recommend performing a push-out test for biomechanical investigations, especially when comparing implants with different structural and surface properties [19].

#### *Implant design for push-out for trabecular bone replacement*

A stiff implant placed in bone will transduce loads and direct these according to its geometry. We felt that a cylindrical implant would at least deflect the direction of load. The study arrangements presented here are based on our previous biomechanical push-out study ([15]). Test implants of the same size with different structural properties (solid vs. porous) were compared with regard to their osseointegrative ability (Fig. 3). They had a height of 7.0 mm and a diameter of 5.6 mm and thus correspond to implants that could be used in humans with related dimensions. Nevertheless, they were basically adapted to the proportions of the implantation site of the host animals (Fig. 3). Furthermore, we provided the implants with a central longitudinal 2.0 mm boring. This enabled the insertion of a 2.0 mm K-wire or mandrel for controlled positioning during implantation, preparation and push-out in mechanical tests (Figs. 4–6).



**Fig. 5.** Positioning of the specimen (star) in the sawing jig. The distal femur was aligned using the central spike wire (arrow) in order to ensure a perpendicular cutting plane to the longitudinal axis of the implant. Finally, the distal femur was covered with gypsum for the further cutting process.

#### *Press fit*

A high initial stability and firm anchoring with direct bone-implant contact implant lead to the desired primary bone healing [28,32]. Micro-movements can prolongate bony ingrowth and eventually lead to early aseptic loosening [13]. According to Schenk, primary bone healing is disturbed with gap widths greater than 1 mm [28]. Primary stability is achieved by the press-fit method, which creates an intimate apposition between bone and implant surface. A minimally undersized implant bed compared to the implant diameter achieves the press-fit implantation [17]. In our previous study, we used a diamond-coated core reamer with a diameter of 5.4 mm, while the implant had a diameter of 5.6 mm ([15]).

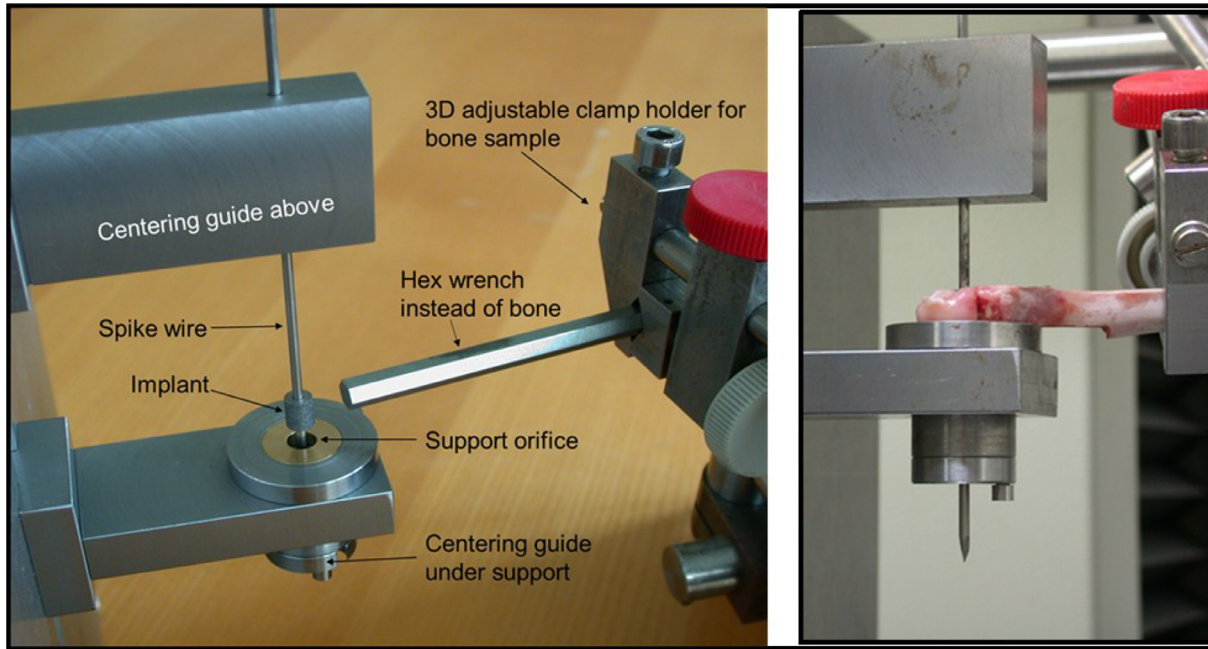
#### *Conformity of axis*

In push-out arrangements, the samples must follow the conformity of the axes exactly to get accurate and comparable results ([15]). Tilt of test implants relative to the axis of symmetry of the driving tool causes a heterogeneous separation of the bone-implant interface, which would lead to incorrectly higher data (“catching effect”) [2]. According to Niki et al. and Li et al., push-out tests often fail to ensure the conformity of the implant axes and the direction of force [23,26]. In this biomechanical arrangement presented, we intended to limit the lateral spread of bone under load and create a restricted situation in order to prevent the bone slab from tilting under load as well as contact of the implant with the edge of the support ring (catching effect) as much as possible ([2,15,19]).

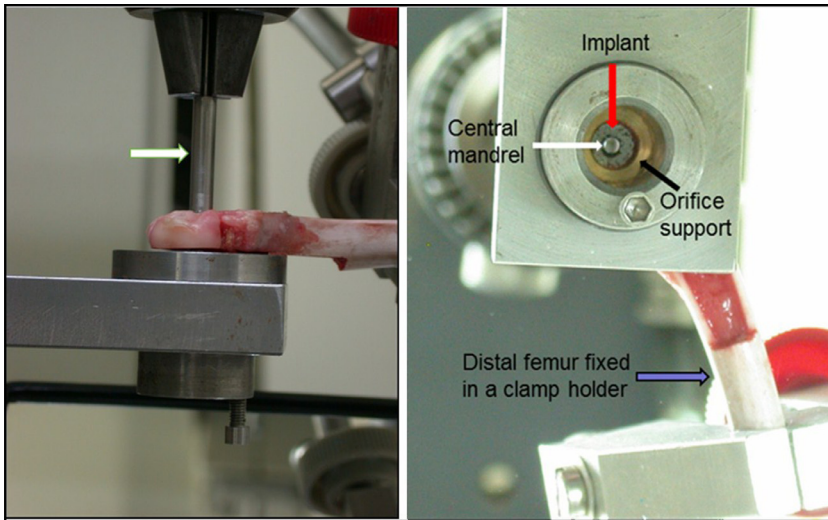
#### *Push-out testing*

#### *Implantation*

The presented implantation protocol is based on the work of Frosch et al. ([15]): Animals received preliminary i. m. anesthesia 0.3 ml (10 mg) Xylazin + 0.5 ml (50 mg) Ketanest)/(kg). Fur surrounding the knees was clean shaven and the skin was disinfected and covered sterile. Continuous infusion of general anesthesia (5 ml Xylazin + 5 ml Ketanest + 40 ml NaCl at 1.7 ml/kg/h) was established using an ear vein. Skin incision over the lateral femoral condyle and exposure of bone proximally of the growth plate allowed preparation of a cylindrical bore. A diamond-coated core reamer (Co. Articomed, Schlüchtern, Germany) with an outer diameter of 5.4 mm was used to reach a depth of 7 mm. Permanent cooling with physiologic saline was applied. The central bone block was extracted and the bottom of the bore was leveled. The implant was carefully centered and press-fit impacted until the lateral side of the implant was as level as possible with the cortical bone (Fig. 1). A porous and a solid cylinder were randomly implanted bilaterally in the distal femora (Fig. 2). Wound



**Fig. 6.** Left: Exemplary test set-up (without implant) for push-out attempts. The spike wire is inserted into the upper and lower centering guide and thus allows the implant to be positioned precisely centered over the orifice of the support. Right: Side view of the experimental setup. The specimen is already positioned over the orifice of the support. For the further push-out process, the spike-wire has to be removed first.



**Fig. 7.** Left: Push-out set-up with specimens centered over the orifice of the support. The push-out device (white arrow) was positioned over the implant just before the push-out.

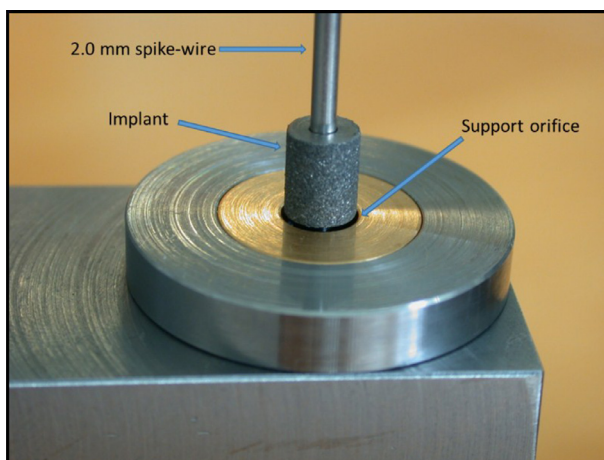
Right: View from under the support. The precisely positioned implant can be seen from below through the orifice of the support. The mandrel of the push-out device is inserted into the central borehole of the implant and enables iso-axial push-out.

closure in layers was followed by an intracutaneous seam and skin surface disinfection. An antibiotic (0.5 ml Penstrep) was given perioperatively once and analgesic (Rimadyl 0.1 ml/kg) the following 3 days. A veterinarian regularly monitored the animals for species-appropriate, pain free behavior and physiologic movement.

#### *Push-out arrangement*

The presented push-out arrangement is based on the work of Frosch et al. ([15]): The femora were harvested with special care not to impair the bone surfaces. After all femora were x-rayed (x-ray standard mammography system, Co. Siemens, Munich, Germany), specimens were shock-frozen by immersion in dry ice cooled iso-pentane and stored at  $-20\text{ }^{\circ}\text{C}$  for later processing [12]. For trimming the specimen were thawed overnight. A close-fitting spike wire, which was introduced into the implants' central boring, centered the femur in a sawing jig before the position was fixed with gypsum (Fig. 5). Sectioning perpendicular to the implant's axis of symmetry (grinding band saw, Messner GmbH; Hamburg, Germany) separated bone from the implant's circular top surfaces and the medial condyle was discarded (Fig. 4). The trimmed femurs were fixed in a custom-made testing frame guided by the center spike wire passing through two temporary centering guides adjusted above and under a support and specimen holder (Fig. 6). After centering the implant precisely over the orifice of the support, the position of the specimen was fixed in an adjustable clamp holder and the two centering guides as well as the spike wire were removed before the push-out testing (Fig. 7). In a Universal Testing Machine (UTM; Zwick GmbH & Co.Kg, Model 1446, Ulm, Germany) a perpendicular iso-axial push-out in latero-medial direction was performed, pushing the implant through a orifice in the support out of its bony implant bed. The push-out device had a central mandrel at the contact area with the implant (Figs. 3, 7) that fits snugly into the central boring of the implants in order to assure an iso-axial push-out. The outer diameter of the push-out device was 4.6 mm, which is undersized compared to the diameter of 5.6 mm of the implants to prevent contact to the bone during push-out (Fig. 3). The diameter of the support orifice was 0.7 mm wider than the outer diameter of the implants (clearance of 0.35 mm) for a controlled separation of the implant-bone interface (Figs. 7,8) [11]. A final position check with a view of the device from below can be carried





**Fig. 8.** Exemplary test set-up. Implant is centered above the orifice. The orifice is 0.7 mm wider than the implant to avoid metal-metal contact.

out with a mirror under the test frame (Fig. 9). The pestle of the UTM applied a preload of 10 N for 30 s followed by linear increasing compression (universal speed of 0.5 mm/min). Penetration of the implant and load force were recorded until a decrease to 80% of the maximum load.

#### Accessories

##### ■ Materials

- ✓ dry ice, iso-pentane, paraffine wax, slow setting gypsum,

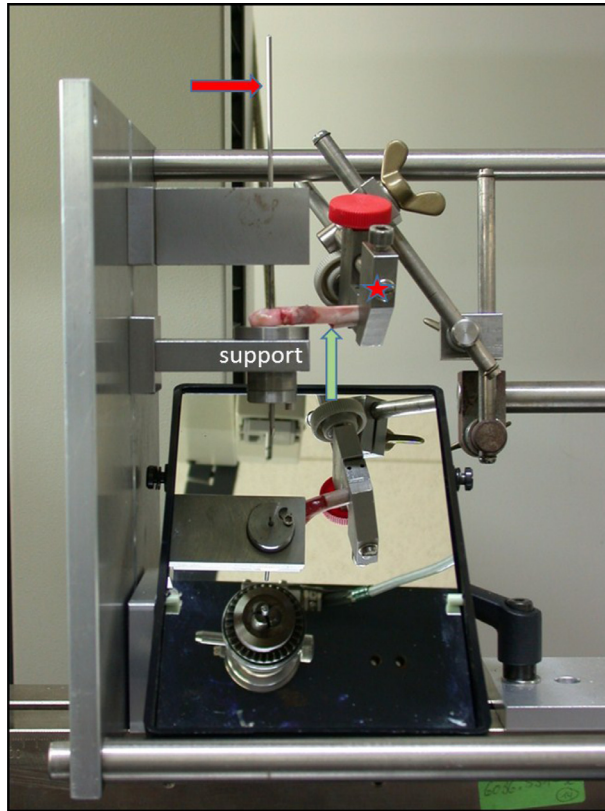
Technovit 9200 embedding material for non-decalcified cutting / sawing of hard plastic

- bediamonded hollow grinder with definite outer diameter,
- metal bowl to be filled with iso-pentane and placed in bed of dry ice
- cutting jig (30× 30 mm square zinc-plated iron tube, two opposing sides reduced by one third, three sets of two borings (diameter 2 mm) precisely opposed and perpendicular to central axis, borings precisely opposed and perpendicular for passage of a tube for passage of screw M8) (Fig. 10)
- 2 mm spike wire for orientation, tube for passage of fixation screw
- rubber beaker for potting in gypsum
- Messner EXACT grinding band saw
- hot stage to melt paraffin wax
- potting jars out of polymer with outer diameter compatible with drill chuck / specimen holder of the Leica diamond inner hole saw
- Leica bediamonded inner hole saw
- Universal testing machine, load cell 500 kN, software for push-out
- Testing frame (cube built out of two square side plates connected by steel rods in the corners, one side plate with centered vertical guiding notch and borings for fixation of upper centralizer and sample table with lower centralizer (Fig. 6 left), spike wire and 3D-flexible clamp to hold and orientate bone sample fixed on opposite side
- distance of the orifice to the outer side of sample table must not exceed the depth of medial cut of lateral femur sample, e.g. 25 mm.
- calibrated orifice (exchangeable brass washer) in reset of sample table

#### Work steps summarized

##### ■ implantation

- ✓ hollow grinder demands thorough guidance to prevent deviation
- ✓ continuous irrigation with saline and drying by suction

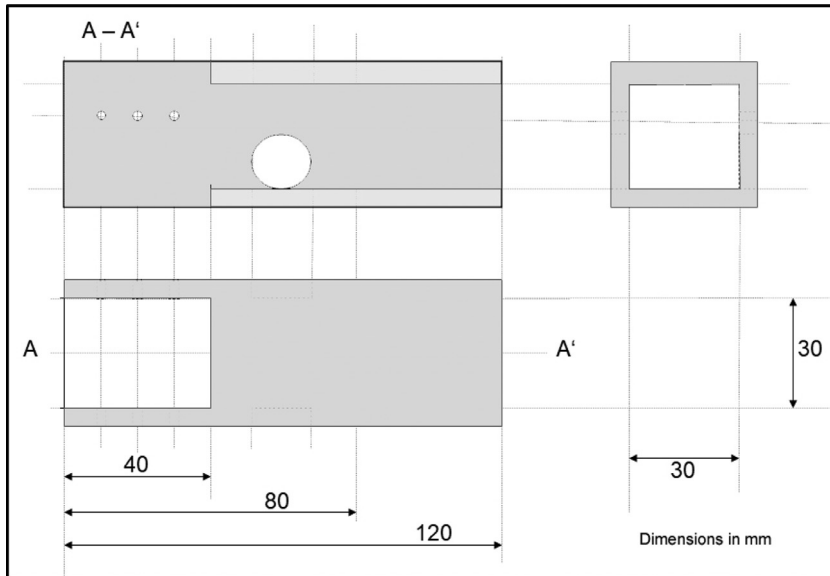


**Fig. 9.** Push-out test frame with a black-rimmed mirror that allows a view from below. The specimen (green arrow) was centered with the spike wire (red arrow) above the orifice of the support and fixed in the adjustable clamp holder (red star)(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

- retrieval of complete femur containing implant,
  - ✓ avoid damage to bone surrounding implant
- take x-ray pictures in two planes
  - ✓ consider MRI/CT small sample examination if artefact reduction is effective
- deep freeze storage (- 20 °C)
  - ✓ use metal bowl with sufficient amount of iso-pentane precooled on dry ice,
  - ✓ submerge the total femur
  - ✓ store femur and cardboard with pencil written identification in two sealing plastic bags.
- Sample preparation
- Push-out examination

#### *Sample preparations*

- repeated submersion of cutting jig in molten paraffine results in 1,2 mm thick coating of lower two thirds of the jig
- preparation for trimming of femur: careful depiction of lateral top surface of implant
- slow penetration of implant's central boring with medium speed turning wood cutting drill, 2 mm diameter
- embedding in slow setting gypsum:
  - ✓ use of cutting jig, spike wire fixation, tube and rubber beaker



**Fig. 10.** Sketch of the cutting jig.

- ✓ centralize the femur with spike wire through borings in implant and sides of jig
- ✓ insertion of tube within the template to allow passage of a fixation screw
- ✓ sink the jig containing the femur in soft gypsum dough for fixation of proximal half of femur with gypsum (centralize proximal femur for complete submersion in gypsum dough, keep jig pressed to the bottom of the beaker) (cf. Fig. 4)
- ✓ after hardening of gypsum removal of spike wire, break cutting jig out of the beaker and remove surrounding gypsum
- fixation of cutting jig on tail stock of the Messner grinding-bandsaw with a screw in the central thread of the tailstock (screw 8 mm diameter, metric thread M8, use of washers to protect jig and tailstock)
- orientate jig horizontally with the distal part of the femur towards the band of the saw
- grind-sawing of antegrade cuts in planes directly next to top surfaces of cylindrical implant (use grinding band saw with repeated cuts at increasing progression of the tail stock to find lateral top surface; ideally no tissue visible on metal surface), cutting depth ca. 30 mm (control corresponding dimension of sample table in push-out frame)
- demount jig from band saw and place the proximal end on hot stage
- melting of paraffin coating on hot stage to free the block of gypsum (place jig in aluminium foil dish to ease cleaning), dissect the femur directly above the gypsum block, use fine saw only (discard proximal half, preserve the tube),
- trimming to expose the circular top surfaces of implant cylinders,
  - ✓ a) cutting off laterally overgrown soft tissue and bone
  - ✓ b) cutting off the medial condyle (Fig. 4)
- selection of samples at random for method 1) push-out or 2) histology, continue trimming in transverse plane
  - ✓ 1) push-out: Preserve the middle diaphysis attached with the lateral condyle for later positioning of sample in clamp
  - ✓ 2) histology: remove bone leaving a 5 mm broad rim of tissue around the implant. The established non-decalcified thin saw-grinding of implants integrated in bone is no further described here.

### *Push-out experiment*

- Centralize testing frame on UTM base under the traverse. Assemble upper centralizer and sample table with lower centralizer,
- use drill chuck under load cell and insert spike wire,
- control friction-free passage of spike wire through both centralizers,
- fixate the proximal end of the specimen (diaphysis) in grip of 3D clamp holder (cf. Fig. 6 left) and strengthen grip of 3D clamps to allow careful orientation of the medial cut surface on top of the sample table,
- lower traverse to let the spike wire pass through upper centralizer, implant's central boring and lower centralizer,
- control position of the specimen with the medial cut surface in plane parallel and close contact with specimen table (cf. Fig. 5 right), control gentle grip of 3D specimen holder clamps.
- under control not to move the sample lift traverse, replace spike wire with mandrel, and remove both centralizers,
- insert tip of mandrel into implant boring (cf. Fig. 6 right), (passage without obstacle guarantees perfect centralisation of implant above the orifice in the support) (cf. Fig. 6 left insert),
- start push-out program

### *Proposal for additional examinations*

- SEM surface inspection of implants pushed out for structures and extent of bone on-growth,
- microradiographs of ground sections for detection and degree of mineralization
- subsequent push-out undecalcified ground sections or SEM inspection of bone tissue once surrounding implants for characterization of fracture mechanism

## **Discussion and conclusion**

The planning of an experiment is probably more important than the implementation itself. The quality of the details and the coordination of actions are decisive and are fundamental to many aspects such as e. g. choice of methods, finances, animal protection, cooperation-partners and -laboratories, availability of investigational tools, host tissues and reactions, time table, properties of materials under research, supporting materials and chemicals and several others. We did not arrange these topics according to importance or time table as every single item may completely endanger the proper conduction of an experiment.

In this presentation, we focus specifically on the selection of the animal model, the biomechanical test arrangements and the technical implementation of the experiment. However, not only the biomechanical examinations but also histology benefit from the concentric positioning. We were able to produce ground sections with perfect circular cross sections of the implants. Trimming of bone for the push out appears to be complicated by the preservation of the femur diaphysis. However, we intended to prevent dislocation of the bone sample after the centralisation over the orifice. Considering the minimum gap between outer surface of the implant and the orifice any deviation should be avoided.

The presented examination protocol enables the assessment of the anchoring strength of the implants as well as the histomorphology and histomorphometry with high reproducibility and precision.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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