Freezing of Rat Tibiae at -20°C Does Not Affect the Mechanical Properties of Intramedullary Bone/Implant-Interface: Brief Report

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Abstract: *Background*: The effects of freezing-thawing cycles on intramedullary bone-implant interfaces have been studied in a rat model in mechanical pull-out tests.

Implants: Twenty TiAl6V4 rods (Ø 0.8 mm, length 10 mm) implanted in rat tibiae

Methods: 10 rats underwent bilateral tibial implantation of titanium rods. At eight weeks, the animals were sacrificed and tibiae harvested for biomechanical testing. Eight tibiae were frozen and stored at -20°C for 14 days, the remaining eight were evaluated immediately post-harvest. Pull-out tests were used to determine maximum force and interfacial shear strength.

Results: There were no significant differences between fresh and those of the frozen-thawed group in maximum force or in interfacial shear strength.

Conclusion: Frozen Storage of rat tibiae containing implants at -20° C has no effects on the biomechanical properties of Bone/ Implant interface.

Keywords: Bone, implant, bone-implant-interface, fixation, freezing/thawing cycles, storage.

1. INTRODUCTION

Orthopaedic and Dental implants rely on osseointegration to achieve mechanical fixation to the host bone, which is a prerequisite for a good clinical outcome [1, 2]. In the past, various approaches have been used to enhance peri-implant bone formation. Hydroxyapatite coatings [3], in combination with growth factors [4, 5], bisphosphonates [6] or biomimetic surfaces [7] have been shown to improve osseointegration of titanium implants. Traditionally, large animal models have been used to study orthopaedic and dental implant fixation [5, 8-10]. More recently, rat models have generated an increased interest [6, 11, 12]. Molecular studies are easy to perform, care and handling of rodents is more manageable and overall costs are reduced. In the rat model a tibial or femoral implant is frequently used to assess osseointegration. The conditions and procedures for all parts of the trial, e. g. anaesthesia, implantation procedures [13], storage of tissue samples and mechanical testing must be defined and replicated at each stage.

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Biomechanical testing has been performed under at least three conditions: fresh explanted bone [3, 5], frozen/thawed bone [4, 13] and dehydrated/rehydrated bone [14, 15]. In many reports, the condition of the bone during testing is not mentioned [6, 16, 17].

The use of fresh bone in biomechanical tests should reveal optimal results, but is logistically difficult, especially when the sample size is large or the biomechanical testing laboratory is at some distance from the surgical laboratory. Additionally, there is a risk of losing specimens, due to unforeseen complications during mechanical testing.

The purpose of the present study was to determine, if freezing and storage of bones with implants at -20° C have any effect on its biomechanical properties utilizing mechanical pull-out tests in a rat model. A modified rat tibial implantation model, described by Gao *et al*. [6] with bilateral implantation of titanium rods was used. We compared the results of mechanical pull out tests of samples which had been stored at -20°C and thawed, with those of fresh explanted tibiae.

2. MATERIALS AND METHODOLOGY

Animals

All experiments were approved by the Animal Care Committee of Thuringia (Reg. No. G 02-008/10). Ten, 3-

months-old male Sprague Dawley rats (Harlan Laboratories GmbH, Eystrup, Germany) weighing 300g –389 g were used. All were given free access to standard rat-chow and water. The environment was air-conditioned with a controlled light cycle of 12 h/dark 12 h, relatively steady temperature and humidity. An institutional guideline for the care and treatment of laboratory animals was followed.

Implants

Twenty custom TiAl6V4 rods (Königsee Implantate GmbH, Aschau, Germany) 0.8 mm in diameter and 10 mm in length were used. All implants were blasted with ceramic beads as a standard procedure for orthopaedic implants.

Sterilization of the implants at 134 °C - 138 °C, 2.16 bar pressure for 35 minutes (Vacuklav 44B, Melag, Berlin, Germany) prior to implantation was performed.

Conditions

Two different conditions were tested:

Group A) Fresh explanted bone with implant *in situ*

Group B) Stored bone with implant *in situ* at -20°C for 14 days

Implant Procedure

Surgery was performed under general anaesthesia by weight-adopted intraperitoneal injection of Domitor® (Meditomidin) 0.15 mg/kg BW(Pfizer, Berlin, Germany), Dormicum® (Midazolam) 2.0 mg/kg BW (Ratiopharm, Ulm, Germany) and Fentanyl® (Fentanyl) 0.005 mg/kg BW (Janssen-Cilag, Neuss, Germany).

Animals were prepared for surgery: Both hind legs were shaved and disinfected with alcohol. Sterile conditions were adhered to throughout the surgery using sterile drapes and sheets. Both hind legs were draped with a sterile incision foil (Raucodrape, Lohmann & Rauscher, Rengsdorf, Germany). A medial incision to expose the knee joint in both hind limbs was made 5 mm longitudinally, and a pilot hole was marked at the intercondylar eminence. A custom-made awl, tip size 0.9 mm in diameter and 10 mm in length was gradually rotated to create a channel from the proximal tibia epiphysis into the medullary canal. The implants were inserted *via* this channel and positioned 2 mm beyond the articulating cartilage. Soft tissue was irrigated with sterile saline with fascia and skin incisions were closed in single-knot technique (Vicryl 5/0 and Prolene 5/0, Ethicon, Norderstedt, Germany). Prophylactic IM antibiotic (Terramycin, Pfizer GmbH, Berlin, Germany) and analgesics (Buprenovet, Bayer, Leverkusen, Germany) were administered once at the time of surgery. Implant position was monitored and confirmed by X-ray. General anaesthesia was reversed by Antisedan® (Atipamezol) 0.75 mg/kg BW (Pfizer, Berlin, Germany), Flumazenil-hameln® (Flumazenil) 0.2 mg/kg BW (Invera Arzneimittel GmbH, Freiburg, Germany) and Naloxon (Naloxon) 0.12 mg/kg BW (Deltaselect GmbH, Dreieich, Germany).

Explant Procedure

The animals were sacrificed at eight weeks. Tibiae were harvested and cleared of all soft tissue. In group A), the tibiae were processed immediately and mechanical testing was completed within 4 hours while samples of group B) were stored at – 20°C for the next 14 days.

Tibia epi- and metaphysis were trimmed to expose the proximal tip of implant for 2 mm - 3 mm by using a bur (Minimot 40IE, Proxxon, Niersbach/Eifel, Germany) to prepare the implant for mechanical testing.

This was carried out in group A) right after explantation; in group B) the samples were frozen, processed and then re-frozen at -20°C.

Biomechanical Testing

Due to one surgical site infection and one anaesthetic complication the sample size was reduced to 8 implants in each group.

The biomechanical property of the bone/implant-interface was assessed using a pull-out test. The distal part of the tibia was embedded in polyester resin (CEM 2000, Cloeren Technology GmbH, Wegberg, Germany), which is selected as it does not develop high temperatures during polymerisation. For embedding, a custom made fixture was used to permit coaxial alignment of the implant in the direction of force (Fig. **1**).

The tibiae were tested in a commercial material testing system **(**Tiratest 2710, Tira GmbH, Schalkau, Germany) post air-thawing at room temperature (Fig. **2**). A distraction speed of 1 mm/min was set for the test, with a load-displacement curve recorded simultaneously. The resultant curves allow a maximum force to be determined and interfacial shear strength then calculated by dividing the force (N) at the point of failure by the surface area of the implant in contact with tissue (mm^2).

Statistical Analysis

Data are presented in Figs. (**1**, **2**) as box-and-whisker plots indicating median, quartiles, whiskers and outliers. StatGraphics Centurion (Statpoint Technologies, Inc., Warrenton, Virginia, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) following multiple comparisons with Fisher's least significant difference (LSD) procedure at the 95.0% confidence level was performed to determine if there were significant differences.

RESULTS

A total of 8 tibiae from each group have been included in this analysis. Biomechanical testing revealed no significant influence of the different conditions (fresh vs. frozen-thawed) on maximum force required to extract the implant or on the corresponding interfacial shear strength (Figs. **3**, **4**).

DISCUSSION

Several studies have been performed to assess the effects of tissue preservation on the mechanical properties of bone in different animal models [18-23]. Usually, mechanical properties of whole bone in 4-point bending tests [20, 21, 23, 24] or of bone slices in compression tests [18, 19] and indentations tests [19] are evaluated. Almost all of these studies have found no significant difference between fresh and frozen samples on mechanical testing [18-21, 23, 24].

Given the large difference in linear thermal expansion coefficients between fresh bone ($89 \pm 2 \times 10^{-6}/K$) [25] and

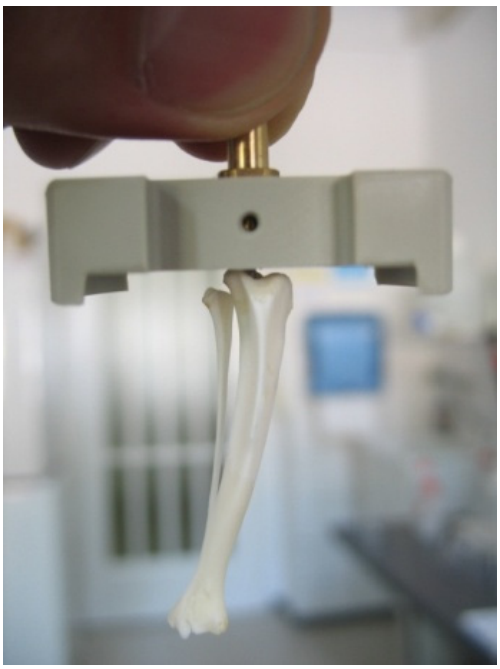

Fig. (1). Photo of the experimental set-up: The implant was fixed in a custom made device, permitting coaxial alignment and then embedded in a polyester resin.

titanium (8.5 x 10^{-6}/K) [26], we hypothesized that a significant difference may exist between fresh and frozen-thawed samples in mechanical testing of bone/implant-interfaces.

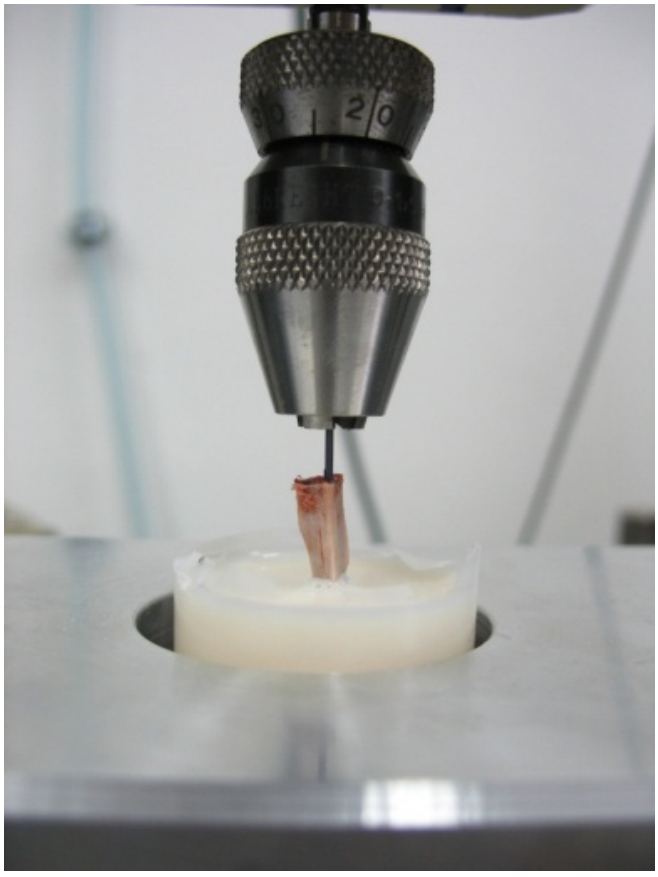

Fig. (2). Mechanical pull out tests: The implant was fixed *via* a three jaw drill chuck to the testing system.

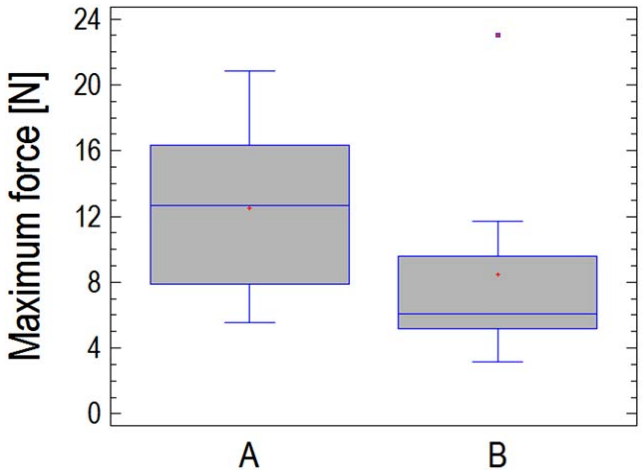

Fig. (3). Maximum force in [N] measured by pull out tests: No statistically significant differences between fresh (Group A) and frozen- thawing (Group B) samples at the 95.0% confidence level (Fisher's least significant difference (LSD) procedure).

We utilized an *in vivo* tibial implant model in rats to analyze the osseointegration of titanium alloy implants. The intramedullary implants are fixed by *de novo* bone formation. This thin bone/implant-interface, which had developed over the eight weeks, may be more susceptible to effects of tissue preservation.

Reports of different preservation techniques in intramedullary implant fixation and mechanical pull-out tests are scarce. Huss BT *et al*. reported no difference in pull-out testing of cortical Steinmann pins in frozen or fresh cadaveric canine bone and also of implants placed in *in vivo* [22]. In our *in vivo* model, we compared fresh to frozen implant-bone interfaces.

Group B (frozen/thawed) showed a trend towards a lower shear strength in the mechanical testing, which was not significant (average shear strength group B 0.45 ± 0.13

N/mm^2 vs. group A 0.63 ± 0.09 N/mm^2). A possible explanation for this trend could be that the bone/implant interface was weakened by the freezing/thawing cycles. No significant difference regarding maximal pull out force and shear strength was found between fresh samples and those stored at -20° and air thawed. These results seem to be in general agreement with the findings on whole bone without implants, despite the bone-implant interface being prone to adverse effects of tissue preservation.

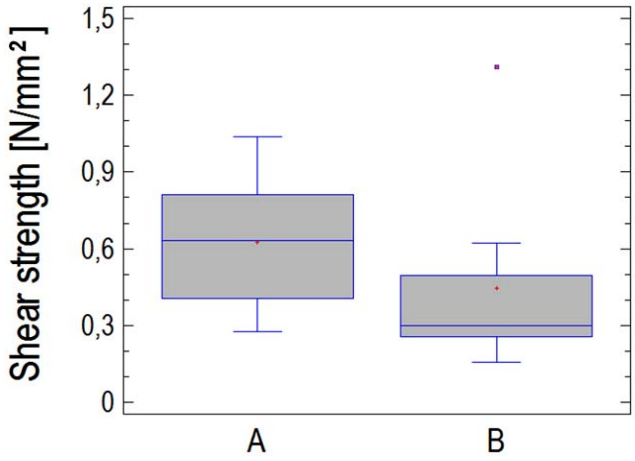

Fig. (4). Shear strength in [N/mm^2] measured by pull out tests: No statistically significant differences between fresh (Group A) and frozen- thawing (Group B) samples at the 95.0% confidence level (Fisher's least significant difference (LSD) procedure).

CONCLUSION

Freezing and thawing does not significantly affect the maximum force and interfacial shear strength in mechanical pull-out tests when evaluating implant fixation in a rat tibial model. Sample storage at -20°C can be used to simplify the experimental set-up.

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