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Schlussbericht BMBF- Verbundvorhaben

„Prävention Implantat-assoziiertes Infektionen mittels innovativer Beschichtung“

Akronym: CUPER

Subproject Nr.4

“Präklinische Untersuchung einer antimikrobiellen
Oberflächentechnologie auf Kupferbasis im Kleintier-
Knochenheilungsmodell“

Teilprojektleiter:

Prof. Dr. Claus-C. Glüer*

* Im Ruhestand, Bericht verfasst von Dr. Sanjay Tiwari



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1. Kurzbericht

1.1 Aufgabenstellung

The coating of intramedullary nails with copper can prevent implant-associated infection in the treatment of fracture repair. It would be desirable for this coating not to impede the initial immune response necessary for effective fracture healing, to possess osteogenic properties, and to prevent the initial inflammatory process from becoming chronic. However, the effect of copper-coating on the fracture healing process is unknown. Due to the complexity of the bone healing process, in-vivo studies are required to determine if surface modification with copper on titanium nails has a potential clinical benefit. In this subproject, the developed surface-coated implant was preclinically investigated over time in a rat bone healing model. The objectives of this small animal study was to analyze implant integration, bone healing under the influence of copper release from the coating, and the mineralization of a bone gap over time periods spanning from the pre-osteotomy state through the initial healing phase to the time at which the fracture has healed. In particular, the following hypotheses will be tested within the framework of this work package.

1.2 Wissenschaftler und technischer Stand

The effect of copper-coating on the fracture healing process is unknown. Serial quantitative micro-computed tomography (q μ CT) as an imaging approach can quantitatively analyze bone changes during the longitudinal course of fracture healing. By precisely registering follow-up images to the baseline image, differentiation between bone formation and bone resorption processes, and in particular, mineralization processes of the fracture callus, can be visualized and analyzed in 3D. Fluorescence molecular tomography (FMT) is an imaging modality which can be used to perform functional imaging. Using biomarkers of inflammation as an imaging probe, inflammatory processes can be detected. The serial measurements can be used to distinguish between positive, short-term inflammations that promote fracture healing and negative, long-term inflammations that are detrimental to fracture healing. These serial images are not established in large animal models, and thus the small animal model complements the large animal model (AP5).

1.3 Ablauf des Vorhabens

An osteotomy was performed in a rat fracture healing model established by the project partner Charité, and the resulting osteotomy gap was fixed with an implant. Subsequently, fracture healing, particularly the mineralization and remodeling of the fracture callus (using q μ CT), and the activity of neutrophil elastase, using the fluorescent imaging marker Neutrophil Elastase 680 (Revvity), as an inflammatory marker (using FMT) was examined over a period of 6 weeks, within which fracture healing should be complete. An uncoated titanium implant was used as a control for the copper-coated titanium implant, and additional rats without osteotomy were studied over time, as a Sham-operated control group.

The study was divided into 4 work packages, WP4.1 to WP4.4, with the individual work steps below.

Work Package	Main Tasks	Duration
4.1	Preparatory work: Ethical application and training.	Mths 1-10
4.2	Adaptation of the image analysis software	Mths 7-20
4.3	Conducting the small animal study	Mths 11-12 and 19-20

1.4 Wesentlichen Ergebnisse

We show by optical imaging that neutrophil elastase activity is approximately 10-fold higher at day 5 in implanted legs compared to non-implanted legs, for both copper-coated and titanium implants. By day 21 the activity has decreased to 2-3-fold for both implants. Longitudinal micro-CT imaging revealed a steady increase in bone micromorphological parameters (Bone Volume/Tissue Volume (BV/TV), Bone Mineral Content (BMC) and Bone Mineral Density (BMD) and Tissue Mineral Content (TMC)) for both implant materials. Tissue Mineral Density (TMD) shows a steady decrease down to around 92% with no significant difference between the groups. TMC vs. TMD are in good agreement with expectations for early mineralization in callus formation. The body weight of the rats for both groups of implants also increased steadily and was similar to the control group. In summary, following an acute inflammatory response, copper-coated implants do not induce chronic inflammation, do not negatively affect the bone healing process and show the same degree of biocompatibility as titanium implants.

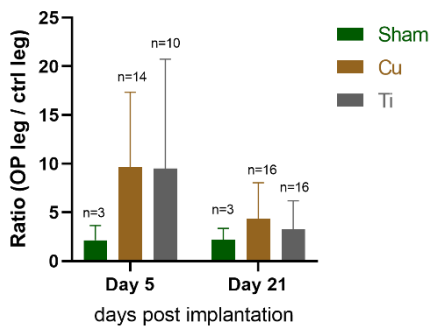


Figure 1. Fold increase in fluorescent intensity of the implant leg over the non-implant leg in Sham operated, test and control groups. The non-implant leg serves as an internal non-inflammatory control. The mean fold increase is less at day 21 compared to day 5, indicating resolution in inflammation.

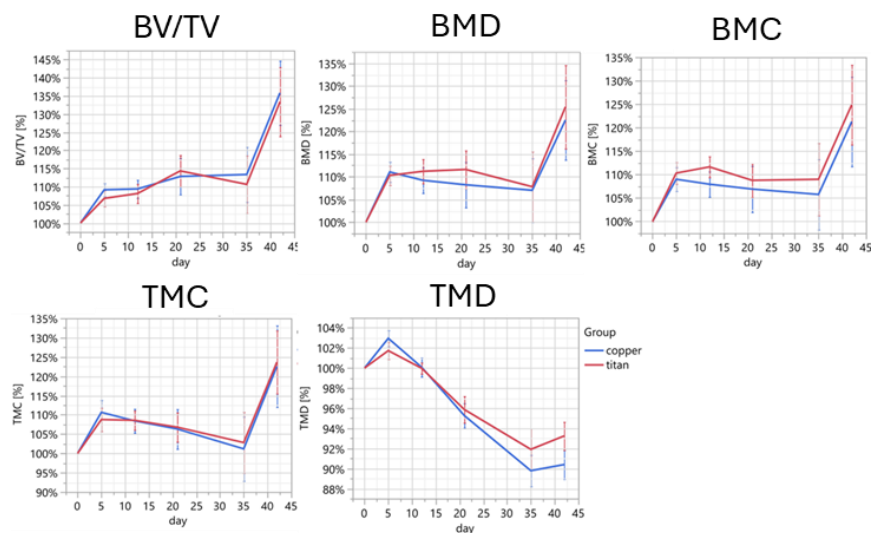


Figure 2. Changes in bone micromorphological parameters measured in rats from Control (Ti) or Test (Cu-coated Ti) groups. No difference in bone micromorphological parameters are observed at any time point. Control (Ti-implant) n=15, Test (Cu-coated Ti implant) n=20.

1.5 Zusammenarbeit

Intermedullary nails consisting of a titanium alloy was purchased by Stryker from RISystems AG and processed by the consortium partner DOT GmbH with an anodization type 2 surface with copper (TEST) or an anodization type 2 surface without copper (CONTROL). The coated nails were then sent to the consortium partner Stryker Trauma GmbH for sterilization and packaging. Specialized orthopedic instrumentation required for the implantation of the test and control articles was provided by Stryker. Training for the insertion of Ti implants (with and without Cu coating, intramedullary nail) into the intramedullary cavity to stabilize the bone and induce femoral fractures in the rat model was given by the consortium partner Julius Wolff Institute, Charité - Universitätsmedizin Berlin.



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1. Eingehende Darstellung des Projekts

1.1 Erzielte Ergebnisse

1.1.1 AP1: Vorbereitende Arbeiten: Tierversuchsantrag, Training und Detailplanung des Operationsprotokolls; Vorbereitung und Detailplanung der Protokolle für die Bildgebung.

The goal of the preparatory phase was to complete and submit an animal testing application, to plan the surgical protocol in detail based on cadaver studies and surgical training, and to finalize the imaging protocols using micro-CT. The milestone (m10) for this work package was achieved. The approval number of the animal ethical application is V242-759757/2021(67-9/21). A training session for the surgical procedure in performing the osteotomy and locking of the intramedullary nail took place at the Julius Wolff Institute, Center für Muskuloskeletale Biomechanik und Regeneration, Charité - Universitätsmedizin Berlin under the leadership of PD Dr. rer. nat. Katharina Schmidt-Bleek. Follow-up training sessions were performed at MOIN CC, UKSH, Kiel. The surgical procedure was performed by Dr Olga Will, a certified veterinary surgeon at MOIN CC. A schematic outline of the surgical procedure is presented in Figure 3 (below).

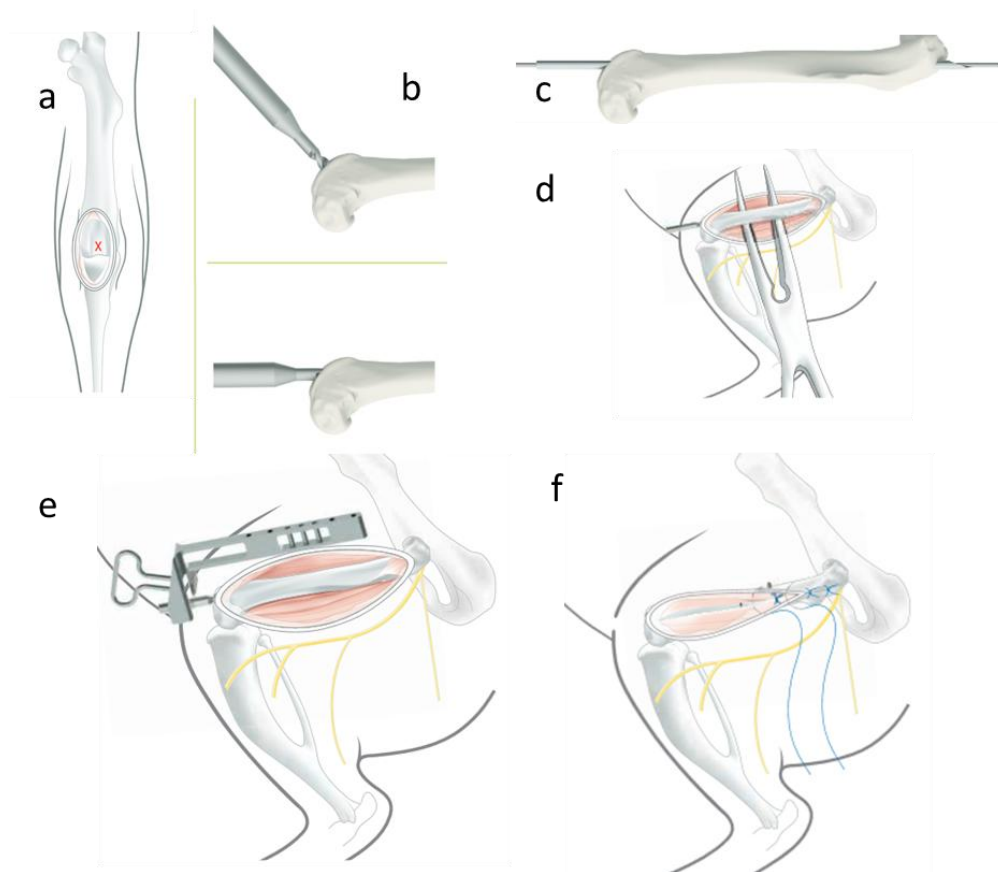


Figure 3: Schematic illustration of the major surgical steps: a) skin incision; b) opening of the femur; c) implant in final position; d) longitudinal skin incision and preparation of the femur for locking of IM nail; e) Mounting of the aiming device; f) suturing

1.1.2 AP2: Adaptation der Bildanalysesoftware

The goal of this work-package is to use micro-CT datasets obtained during the preparatory phase to adapt image analysis software already developed at MOIN CC to the requirements of the COVER study, namely development of valid algorithms for assessing bone mineralization.

For the purpose of optimizing the micro-CT imaging protocol and to reduce variability in longitudinal measurements, osteotomy was performed on 2 rats (OP date 1.12.2021), one which received a titanium implant and one with a copper implant. Imaging was performed according to the schedule outlined in figure 4 below.

Gruppe 21 Tage

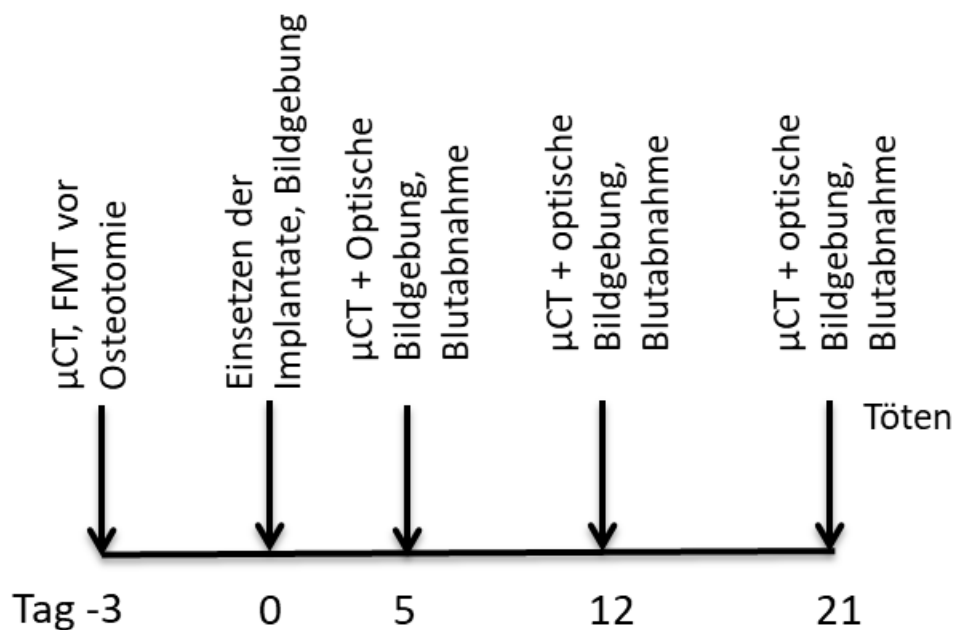


Figure 4. Imaging using micro-CT and FMT was performed prior to Osteotomy at day -3. A quick micro-CT scan was performed following insertion of the implant nails to ensure bi-cortical penetration of the nails. Subsequent imaging days with micro-CT and FMT were on day 5, day 12 and day 21 following implantation.

Micro-CT scans were acquired prior to osteotomy and immediately postoperatively and at 5, 12, and 21 days after surgery (Viva80CT micro-CT device, SCANCO Medical AG, Bruettisellen, Switzerland). The scanned region was 8,5 mm long, centered around the fracture gap, with a \varnothing 80 mm field of view. The image quality and reduction in variability in longitudinal imaging was achieved through 3 modifications.

- 1) Image quality directly around the titanium implant was improved using a beam-hardening correction as part of the standard SCANCO micro-CT reconstruction and replacing the standard aluminum filter with a 0.1mm copper filter.
- 2) To reduce artifacts associated with rat leg twitching that occurred in anesthetized rats, a 3D printed femoral fixation device was fabricated that allowed stable positioning of the rat femur. The developed holder pulls the proximal tibia just below the knee joint upwards using

adhesive strips to an adjustable plastic surface (blue in Figure 5A.), to which it rests securely. The foot of the same leg is also fixed to the edge of the rat holder used with adhesive strips. As a result, the knee is decoupled from movements and is also protected from slipping over the course of the scan. The femur can therefore be considered to be fixed as best as possible by its positioning between the knee and hip joints. Furthermore, the positioning allows for the alignment of the femur along the CT scanner axis, which improves overall CT imaging of dense metal implants and simplifies the analysis of the same region of the femur in rats. In addition, the device was covered with diaper material to eliminate artifacts due to excreted urine.

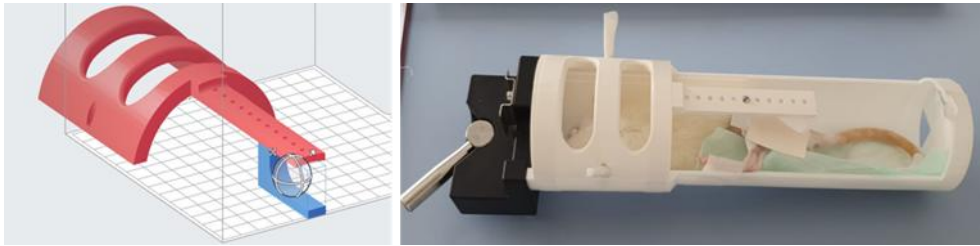


Figure 5. A. Schematic image of 3D-printed leg fixation device B. Photo of rat placed in the micro-CT holder using the 3D-printed leg fixation device.

- 3) StructuralInsight is a software package that has been developed over many years in the Biomedical Imaging Section, which allows the individual adaptation of quantitative image enhancement methods to the needs of the respective study. The region of analyses is the fracture gap, delineated following an automatic alignment of the bone implant nail from the micro-CT scan and a semi-automatic determination of the position of the fracture gap (Figure 6A&B). An evaluation region displayed in blue is then automatically generated which excludes the implant material (frac gap') (Figure 6C). A second evaluation was performed with the evaluation slice including the implant material in the center ("integral").

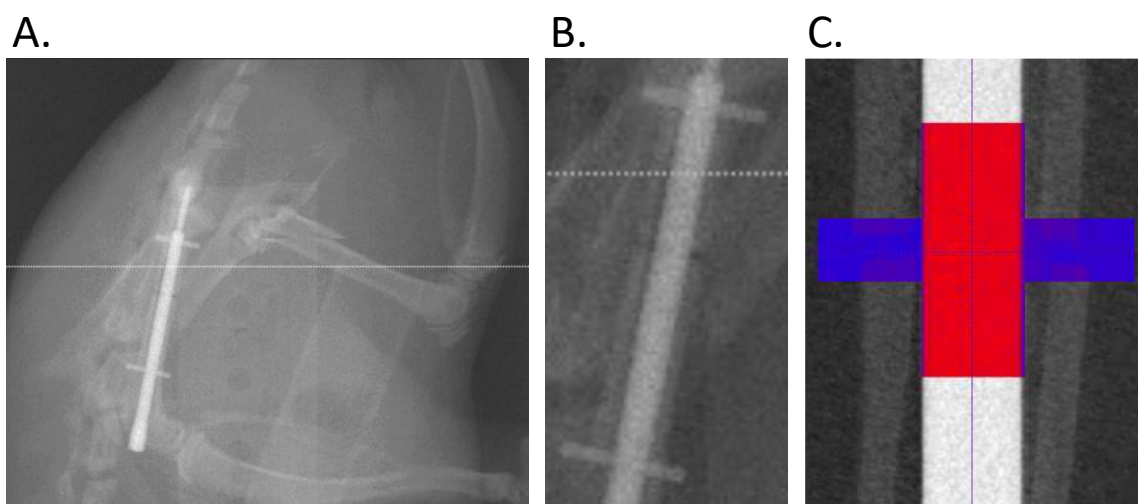


Figure 6. (A) Micro-CT scan to establish the evaluation region. (B). The data is automatically aligned based on the bone implant nail. (C). After semi-automatic determination of the position of the fracture gap, the blue evaluation region is automatically generated which excludes the implant material (frac gap' analyses), but the ROI includes the endocortical bone adjacent to the material, the

cortical bone and the callus. Evaluation with a ROI which includes the implant material in the slice (red colored) was also performed (integral' analyses).

1.1.3 AP 4.3: Durchführung der Kleintierstudie

The goal of this work-package is to monitor the welfare of the animals following osteotomy and ensure the imaging data obtained in the longitudinal studies of 21 days and 42 days, are accurate readouts of the effect of the implant and not influenced by associated co-morbidities.

Three groups of 20 animals (sham, control and test group) were planned for a two-part study. The first part (10 animals per group) was planned to last until 21 days post-implant. After evaluation of results from part 1, part 2 of the study (10 animals per group), which was planned to last until 42 days post-implant, could commence. The SHAM group has been included in order to measure the inflammatory response as a consequence of the surgical procedure without implantation

The schedule of day 21 and day 42 studies are outlined below in figure 7.

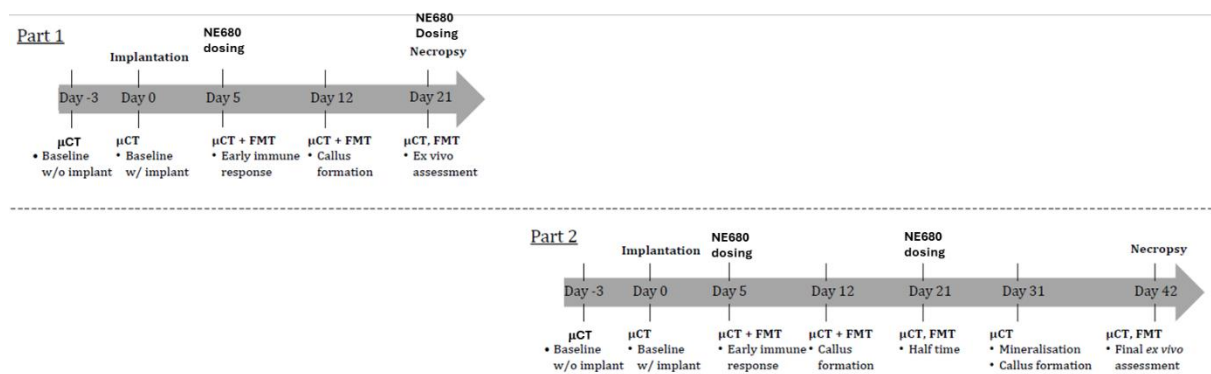


Figure 7. Schedule and imaging of bone micromorphology by micro-CT and fluorescent inflammation marker NE680.

The animals were examined daily after the surgery for 4 days and then at every imaging point (days 5, 12, 21, 31, 42) to detect mortality, morbidity or any abnormal clinical events. During this examination, the following parameters were documented: weight, activity score, duration of imaging procedure, additional comments.

Animals were withdrawn from the study if they showed the following signs/ratings:

- Weight loss >20%
- Significant pain with strong redness or swelling at implantation site.
- Little interaction with other rats and apathy in spontaneous behavior
- Shaggy fur in conjunction with red eyes
- Rat cannot move
- No drinking or eating over a 24h observation period
- Convulsion
- Massive bleeding during/after surgery

Table 1 lists the actual number of animals operated on and included in the study

Table 1. Planned and actual rats used and analyzed for each of the three groups

PART 1	Test	Control	Sham
Planned	10	10	10
Actual-operated	16	14	4
Excluded - welfare	3	6	0
Excluded - PIN dislodged	4	3	0
Actual Analysed	9	5	0

PART 2	Test	Control	Sham
Planned	10	10	10
Actual-operated	16	10	0
Excluded - welfare	2	0	0
Excluded - PIN dislodged	3	0	0
Actual Analysed	11	10	0

Twenty-one animals in total were excluded and sacrificed from the study for welfare reasons or due to a dislocation of the pin during the recovery period. Two of the rats which were excluded for welfare reasons featured swelling of knee/joint region. Other reasons included: weight loss greater than 20% (n=1), fibrinous peritonitis (n=4), gastric obstruction (n=1), intestinal wall hernia (n=1), fecal impaction (n=1), anesthesia (n=1) All rats excluded from the study were sacrificed immediately. All other animals recovered very quickly after the operation; only 18 hours later they were able to carefully place weight on their legs. After 48h the animals were able to jump in the cage, were agile and show species-typical behavior. On day 21 the incision had healed completely. Upon sacrifice, no obvious discoloring of the soft tissue around the implant was observed. Subcutaneous exposure of the operated area revealed closure of the subcutaneous fascia as well as the muscle layers overlying the femur (Figure 8). No wound hematoma or infection was observed.

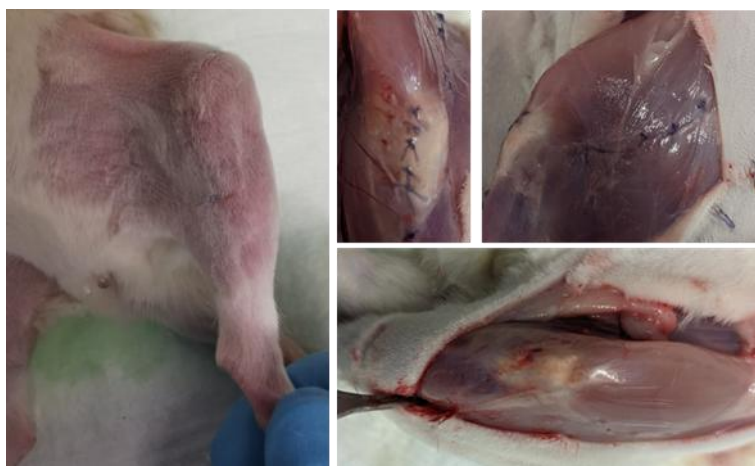
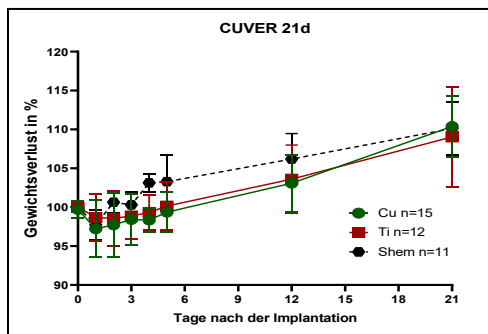


Figure 8. Representative images of the surgical area 21 days after femoral nail implantation. The animal was euthanized after the last imaging. The images show a well-healed knee joint and thigh muscles, which had to be separated due to locking of the intramedullary nail.

No differences in body weight were observed over a 21-day period (Figure 9A) or a 42-day period (Figure 9B) between rats implanted with the cu-coated Ti implants (test implant) and rats implanted with Ti implants (control implant). Since weight loss is a clear sign of excessive toxicity, while slower

body weight growth compared to control animals may suggest mild toxicity, the study further supports the hypothesis that copper-coated implant does not induce toxicity.

A.



B.

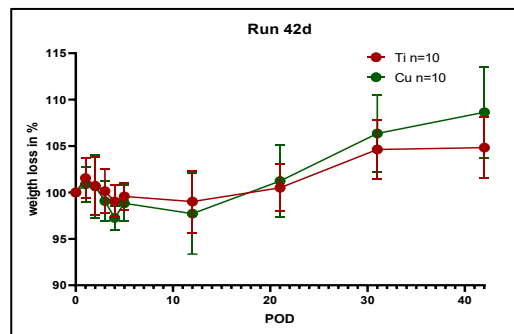


Figure 9. (A) Changes in body weight in test (Cu-coated Ti implant), control (Ti-implant), and sham groups (surgery without implant) over a (A). 21-day period and (B). 42-day period. No statistical difference in weight over both time periods was observed between the groups.

1.1.4 AP4.4 Analyse, Bewertung und Dokumentation der Ergebnisse

The goal of this work-package is to analyze, evaluate and document the data from the animal study in order to draw a conclusion on whether the results support or reject the hypothesis of the study., namely:

- A copper-containing coating with concentrations that have an antimicrobial effect does not negatively affect the bone healing process
- When using copper-containing coatings, no long-term inflammatory process occurs.

The following micro-morphological parameters were analyzed:

- Bone Volume Fraction (BV/TV), assess bone mass but no account of mineralization
- Bone Mineral Density: The amount of mineral content per unit of bone volume which may also include non-tissues (mg HA/cm³). It represents the mineralization level of the bone matrix.
- Bone Mineral Content: The absolute quantity of mineral exclusively in bone (mg HA). It's a measure of bone mass and is a subset of TMC.
- Tissue Mineral Density: The amount of mineral content per unit volume of the mineralized tissue only. A measure of tissue quality (degree of mineralization, mg HA/cm³)
- Tissue Mineral Content: Total mineral content in a given volume of mineralized tissue, which might include tissues other than bone, such as calcified cartilage or other mineralized structures (mg HA).

The fracture gap (Frac Gap) region of the implant was analyzed, and a mask layered over the implant material excluded the implant material in the analysis. The Frac Gap region is the region where reparative processes following a fracture occur. The region encompasses the formation of a soft callus of cartilage which is subsequently replaced with a hard callus of bone. Frac Gap analyses for the individual bone parameters are presented as the percentage change over time (Figure 10), which has the advantage that baseline variations are masked out from the statistics. The data indicate that

(1) BV/TV, BMC and BMD (all threshold-free) and TMC show a steady increase for both test and control group reaching around 125% after 42 days with an intermittent plateau at around 110%. (2) TMD shows a steady decrease down to around 92%. This likely reflects the immature mineral matrix in the newly formed callus, which is amorphous and more translucent to X-Ray photons. (3) TMC vs. TMD are in good agreement reflecting callus formation with reduced amount and density of mature mineralized crystalline hydroxyapatite in the mineralized tissue. The data indicates that during the acute inflammatory phase, there was an increase in bone volume and mineralization, which then plateaued during the healing phase. After day 35, the fraction of highly mineralized bone continued to increase until the study endpoint. At no timepoint was there any significant difference in the parameters examined between the Control group and the Test group.

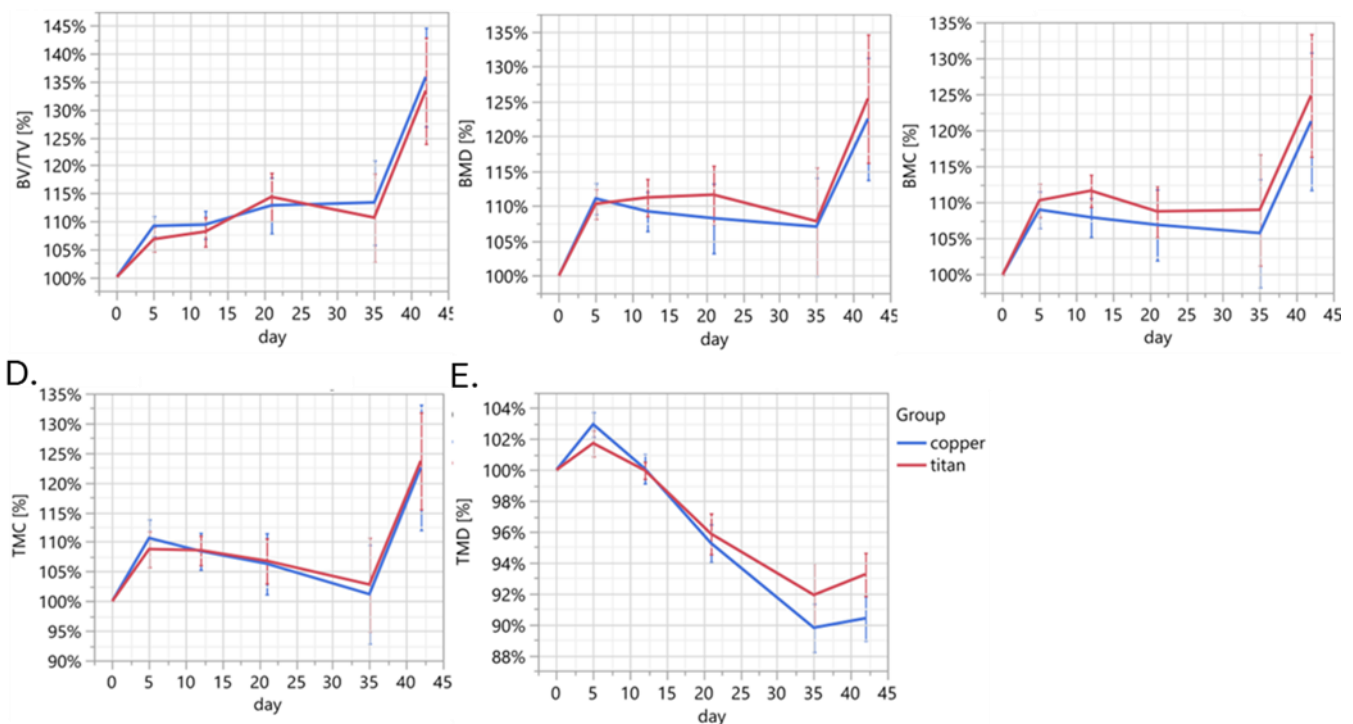


Figure 10. Changes in bone micromorphological parameters measured in rats from Control (Ti) or Test (Cu-coated Ti) groups. Analyses were performed in the fracture gap (frac gap) region which excluded the implant material. Relative changes from baseline day 0 values, prior to implant, are depicted. Changes in (A). BV/TV (B). BMD (C). BMC (D). TMC and (E). TMD are similar for both Control and Test groups and no difference in bone micromorphological parameters is observed at any time point. Control (Ti-implant) n=15, Test (Cu-coated Ti implant) n=20.

Neutrophil elastase activity

Optical imaging using Neutrophil Elastase 680 (NE680, Revvity) revealed stronger mean fluorescent signal and therefore higher neutrophil elastase activity at all time points in the implanted leg (surgery) compared to the non-implanted (control) leg. No significant differences in mean fluorescent signal were determined between the test and control groups (Figure 11). However, the increase in fluorescent signal in the non-implanted (control) leg on day 12, following dosing at day 5, was unexpected since the non-implanted leg is not inflamed. This likely indicates that there is a certain amount of probe which continues to become activated and emit a fluorescent signal independent of

inflammatory activity. Therefore, analyses were reperformed using the signal from the non-implanted leg as the baseline.

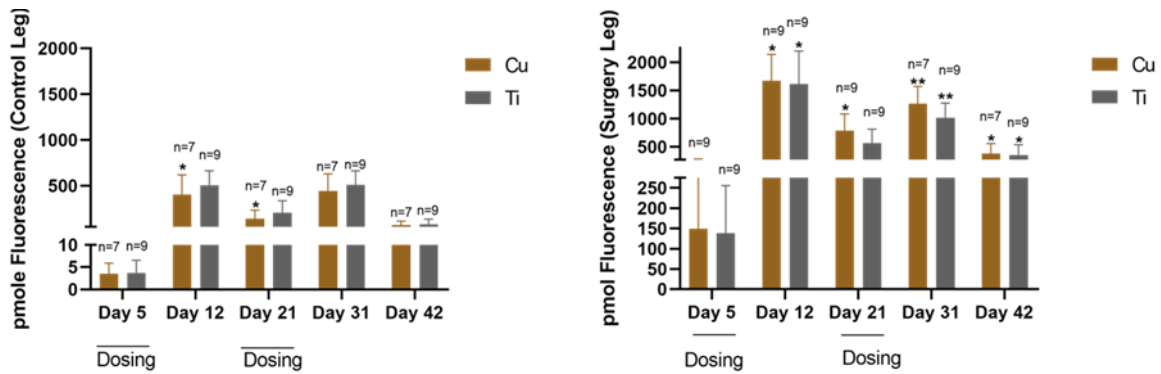


Figure 11. (A). Fluorescence intensity emitted from the optical imaging probe NE680 FAST as a measure of neutrophil elastase activity in wildtype, control and test groups. The imaging probe was administered on day 5 and day 21 post-implant. Fluorescent measurements were performed at the times indicated. (A) legs without implant and (B). Legs with implant. Picomole fluorescence \pm SD, * $p < 0.05$, ** $p < 0.01$ compared to wildtype. There is no statistical difference in neutrophil elastase activity between control and test groups up to 42 days post-implant. Mean \pm SD

The increase in fluorescent signal in the implanted leg is now expressed as a ratio of fold increase over the control leg signal (Figure 12). A Sham operated group was included in the analyses to assess whether the inflammatory response of the implant leg was stronger than the inflammation response induced by surgery. The data indicate that at day 5, the mean fluorescence intensity is 10-fold greater in the implanted legs compared to non-implanted control legs. In contrast Sham operated legs have a 2-fold greater intensity. Furthermore, the fold-increase decreases at day 21 in the implanted legs to around 2-3 fold. The ratios for both Cu-coated and Ti groups were similar at both time points (Figure 12). Therefore, it is imperative that relative fluorescent signals, determined as a ratio with the non-implanted leg, are obtained to understand if inflammation in the implanted leg is increasing or resolving.

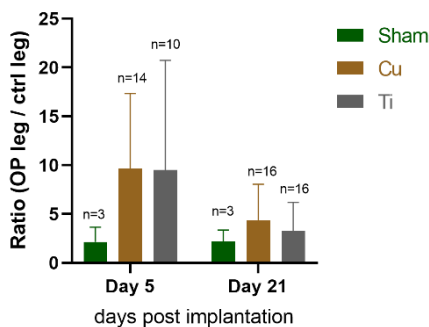


Figure 12. Fold increase in fluorescent intensity of the implant leg over the non-implant leg in Sham operated, test and control groups. The non-implant leg serves as an internal non-inflammatory control. There is a mean fold increase for both implant groups compared to the sham operated group at day 5, albeit not statistically significant. The mean fold increase is less at day 21 compared to day 5, indicating resolution in inflammation.

Taken together, the study confirmed the hypothesis that the copper-containing coating with concentrations that have an antimicrobial effect does not negatively affect the bone healing process and secondly when using copper-containing coatings, no long-term inflammatory process occurs. The following reasons are given for the confirmation of the hypothesis.

- 1) Weight loss is a clear sign of excessive toxicity, while slower body weight growth compared to control animals may suggest mild toxicity. No differences in body weight were observed between animals with copper-coated implants and those with titanium implants, indicating that the copper-coated implant does not induce toxicity.
- 2) The polymorphonuclear response to a foreign body is designed to establish an acute inflammatory environment to contribute to hematoma formation and clearing of debris material. Importantly, a timely acute inflammatory response should be resolved in the first 7 days, otherwise chronic inflammation could lead to fibrotic encapsulation. Poor biocompatibility of implant material often leads to fibrous encapsulation. The measurement of neutrophil elastase activity, secreted by neutrophils during inflammation, is an indirect measure of neutrophil recruitment to the implant site. The decrease in neutrophil elastase activity from day 5 to day 21 indicates that inflammation is resolved or resolving.
- 3) Following the acute inflammatory phase, a reparative phase begins in which soft callus of cartilage is formed and subsequently replaced with a hard callus of bone. The final phase in fracture repair is the remodeling phase which begins around 3-weeks, whereby bone resorption activity is tightly linked to bone formation activity to reestablish bone anatomy and physiology. By serial micro-CT imaging we were able to observe a continual increase in bone volume fraction, indicating increasing periosteal callus volume. Mature bone matrix developed from the Immature callous mineralization occurred from day 35.

1.2 Wichtigste Positionen des zahlenmäßigen Nachweises

The numerical evidence for the project reflects, in large, the structured and targeted use of granted resources in alignment with project goals. The highest expenditure category was the material costs (0843), a sum of 48.158,97 €. This included animal costs, scan time costs for micro-CT and FMT, as well costs of the inflammation markers. Additional costs were incurred due to one group of animals becoming sick and the experiment had to be terminated and repeated. This led to an additional cost of 1.045 € for the extra rats and an additional 5900 € for the inflammation marker. A reallocation of funds from the scan-time costs and travel costs was applied for, to cover the shortfall in this category. The second highest category was the personal costs (0812) of 39.438,35 €. The project was technically demanding and required personnel with varying expertise, including a veterinarian, a scientist with expertise in computed tomography, and a scientist with expertise in optical imaging. Furthermore, student assistance was utilized to perform time-consuming jobs such as creating Region of Interest contours to analyze scans and assisting the veterinarian.

1.3 Notwendigkeit und Angemessenheit der geleisten Arbeit

The general incidence of surgical site infections after fracture fixation is as high as 30% in open or high-risk fractures (1). Surgical site infections can lead to implant failure, multiple surgeries and prolonged antibiotic treatment and in general are significant healthcare costs with increased morbidity. Copper-coated titanium implants offer a promising and safe treatment to fractures which can potentially prevent surgical site infections, but the use of copper is only known from basic research and has not yet been transferred to clinical application (2-4). According to the regulatory framework for medical devices (EU MDR 2017/745), preclinical data is required to test biocompatibility and functional testing in vivo to gain a better understanding of the technology and to ensure its effectiveness and safety before its final application in the patient. For functional testing of intramedullary nails, use of a fracture model is most appropriate, and comparisons should be made between uncoated and copper-coated nails. Amongst the safety considerations are copper toxicity and the impact on bone healing.

The work packages in this project were essential to address safety and functional testing in-vivo. We utilized a rat fracture model and compared titanium implants versus titanium implants with copper coating. We addressed weight changes over time as a biomarker of systemic toxicity, changes in bone morphological parameters during fracture repair as biomarkers of bone healing and neutrophil activity as a marker of chronic inflammation and biocompatibility. We used sufficient number of rats (n=15 in control (Ti), n=20 in test) (Cu-Ti), to ensure statistical power in the data obtained. The project was labor intensive, with repeated measurements performed over a period of 42 days and a vast amount of data accumulated which needed to be analyzed and evaluated. We enlisted experienced scientists in the project, who have had experience with in-vivo studies and data analyses in their specialty (veterinarian, CT expert, Optical Imaging expert). Overall, the work packages were well aligned to the resources used (staff, time and funds) and proportionate to the goals of the project and the funding.

1.4 Voraussichtlicher Nutzen und Verwertbarkeit

The immediate beneficiaries from the results are both Stryker Trauma GmbH and DOT, who have a contractual relationship to enable the future translation of this innovative coating technology up to market readiness/clinical practice. We have submitted a final version of a Preclinical Investigation Report, which is essential for obtaining regulatory approval of a novel medical device. In this report we have presented scientific data in support of copper-coated nails to be safe, biocompatible and functionally effective in an animal model. Ultimately, Stryker Trauma GmbH and DOT can exploit the results to bring a new product into clinical application.

For MOIN CC, we benefit from the knowledge and experience gained in this study. We have developed expertise and know-how in the rat fracture model, in reducing artifacts in micro-CT scans induced by the implant material, and in quantifying neutrophil activity in-vivo. We can apply the collective expertise in early-stage screening in small animals of other materials, for proof-of-concept studies and for justification for moving to clinical use. Furthermore, the micro-CT techniques developed in this project can also be transferred to CT examinations using human scanners at the University Hospital Kiel. We can also anticipate closer collaboration with the Helmholtz Center Hereon in Geesthacht, who have established a permanent branch at the MOIN CC and have developed novel resorbable implant material. Ultimately, as a DFG-registered imaging core-facility, it is important that we publish our optimized methods and disseminate the expertise at conferences in order to attract new customers from industry and new collaboration with academic partners, including grant applications for follow-up funding.

1.5 Fortschritt auf dem Vorhabensgebiet bei anderen Stellen

During the course of the project, a relevant development in the field of measuring bone mineralization in bones harboring an implant was the application of artificial intelligence to calculate implant artifacts (5). The 2023 study validated a deep-learning-powered metal artifact correction (AI-MAC) algorithm using clinical CT scans with orthopedic screws. It showed reduced artifact extent, enhanced signal-to-noise ratio, and improved visualization of vertebral bone, outperforming both traditional metal artifact reduction and virtual monochromatic imaging. This effectively allows for more reliable measurements of bone near implants—critical for BMD estimation. Deploying such AI pipelines in small-animal micro-CT models could enhance research quality—offering automated analysis of peri-implant trabecular structure and densitometry. If AI can be used to reduce implant artifacts, this would strengthen the profile of MOIN CC as an (AI) platform for preclinical studies. Successful AI algorithms could then be transferred to human imaging (transfer learning) in cooperation with radiologists at the UKSH, where they could also improve image quality.

1.6 Erfolgte und geplante Veröffentlichungen und Vorträge

Two papers are planned. The first is on the application of the Neutrophil Elastase 680 optical imaging probe, purchased from Revvity. This imaging probe has previously been used in preclinical studies in mouse models of acute lung injury, arthritis and atherosclerosis. The manuscript will be the first to demonstrate the probes applicability in inflammation induced following implantation of a foreign body.

A second manuscript will be focused on the approaches taken to optimize image quality of micro-CT scans in small animals with metal implants in a bone fracture model. The approaches used, namely beam-hardening correction, replacing the standard aluminum filter with a 0.1mm copper filter, mask

layering of the implant region and the 3D-printing of a femoral fixation device to reduce artifacts associated with rat leg twitching and femur alignment.

2. Literature

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