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RESEARCH ARTICLE

# Evaluation of the Tobacco Heating System (THS) During Closed Lower Limb Fracture Healing in Trauma Smokers' Patients

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## Abstract

**Background:** Since 1976, it's been recognized that increased cigarette consumption correlates with decreased bone density, hindering fracture healing and leading to prolonged hospitalization. Although prior research has shown the relatively less harmful effects of electronic nicotine delivery systems (ENDS) on bone cells in lab settings and animal models, clinical evidence regarding their impact on fracture healing remains scarce. This study aims to investigate whether switching to a tobacco heating system (THS) post-orthopedic surgery improves outcomes for smoking patients during tibia or femur fracture healing over a 6-month period.

**Methods:** The study is a prospective, open-label, non-parallel, single-center trial involving 150 patients from a Level 1 Trauma center, Germany, diagnosed and treated for closed tibia, closed femur shaft, or closed distal femur fractures (according to AO/OTA: 41A2-41C3, 42A-C, 43A-C, 32A-C, 33A2-3, 33B-C). Participants will be categorized into three groups based on smoking behavior: smokers (no intervention), THS (participants switching from cigarettes to THS), and ex-smokers (participants abstaining from cigarettes or ENDS during the study). Clinical, radiological, and laboratory data will be collected during preoperative and postoperative assessments at 6, 12, 18, and 24 weeks. The primary outcome will be the serum concentration of N-terminal propeptide procollagen type 1, a bone formation marker. Secondary outcomes include bone metabolism, healing, immunological, blood count, and clinical parameters. Approval for the study protocol and consent declarations was obtained from the ethics committee of the medical faculty of Eberhard Karls University (724/2022BO1).

**Discussion:** The study results will provide evidence that switching to THS previous orthopedic intervention improves clinical outcomes during closed tibia or femur fracture healing in smokers' patients due to reduced bone resorption rate consequent to the diminished activity of cigarette smoke-activated osteoclast.

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## 1. Introduction

According to the World Health Organization, due to cigarette smoke (CS), 8 million deaths per year will occur in 2030[1]. In 2015, nearly 1 billion people smoked worldwide[2]. CS represents a major health risk that affects the entire human body and is linked to several health conditions (*e.g.*, coronary heart disease, chronic obstructive pulmonary disease, cerebrovascular disease, and cancer)[3][4][5][6]. Moreover, CS is a risk factor for impaired bone homeostasis, resulting in secondary osteoporosis and associated bone fractures, osteoarthritis, and correlates with an increased risk of post-surgical complications such as delayed or impaired bone healing and infections[7][8][9][10][11][12][13][14].

Osteoblasts (bone-forming) and osteoclasts (bone-resorbing) are the central bone cells involved in maintaining the constant equilibrium of bone tissue, and these cells also play a crucial role during the reparative phase of bone fracture healing[15][16]. A fracture occurs when the continuity of the bone tissue is disrupted due to high-force impact, stress or other medical conditions *(e.g.*, osteoporosis, cancer, or osteogenesis imperfecta). Bone fractures are the most common reason for orthopedic trauma surgery.

Since 1976, several studies have demonstrated a positive association between the number of cigarettes consumed and reduced bone tissue mass[17][18][19]. Moreover, CS not only increases the risk of delayed fracture healing[20], non-union[9], and complications[21] but also leads to longer hospital stays[8][9][22][23][24]. Based on clinical observations, the risk of non-union after ankle arthrodesis increased 3.75-fold in smokers[25]. It was shown that in the first 1-2 years after two-level laminectomy, 40% of smokers developed non-union, while only 4% of non-smokers developed non-union[26]. Additionally, smokers undergoing orthopedic surgeries experienced a higher risk of postoperative complications (*e.g.;* infections, implant revisions) than non-smokers[23][27]. Delays in fracture healing, non-union, an increased complication rate, and extended hospital stays increase health system costs. Therefore, developing alternatives for smoking orthopedic trauma patients that improve bone healing are strongly needed.

Our previous study confirmed that CS is a major risk factor for complications such as infection, delayed healing, and revision surgery in orthopedic patients from a Level 1 Trauma center[24]. Unexpectedly, our orthopedic patients who smoke were, on average, 5.4 years younger than non-smokers, demonstrating the harmful effect of smoking on bone quality, with a high risk of bone fracture at younger ages[24]. This finding supports the lower bone quality for young smokers reported by Rudang *et al.*[7]. Additionally, our study showed the immunosuppression status of smokers (reduced levels of pro-inflammatory markers [*e.g.* IL-1β, IL-6, and TNF-α])[24]. This reduction is consistent with the already reported increased risk of infection in smokers compared with non-smokers[28][29]. This is in line with other reports[9][30][31].

Cigarette smoke contain more than 6,000 different molecules, of which toxicity has already been proven for more than 150[32][33]. Nicotine is the most pharmacologically active component of tobacco smoke. Its effect on the proliferation and differentiation of mesenchymal stem cells, which play an essential role in fracture healing through migration and osteogenesis, has long been the subject of controversy. Depending on the dose, both positive[34][35] and negative effects[36][37] have been demonstrated. In 2018, our working group showed that nicotine and its most important metabolite cotinine have no direct effect on the osteogenetic differentiation of mesenchymal stem cells at physiological

concentrations[38]. These results are in line with clinical studies that demonstrated a reduced complication rate after orthopedic trauma surgery for patients with non-electronic nicotine replacement therapies compared to smokers[30][39]. Hence, it can be hypothesized that the harmful effects of CS are related to the molecules produced by the combustion of tobacco.

Quitting smoking is the most effective method to reduce the detrimental effects of cigarette smoke on the human body[21]. Several studies demonstrated that cigarette smoking abstinence pre-orthopedic intervention reduces postoperative complications[40][41]. Moreover, smoking abstinence with non-electronic nicotine replacement therapies (*e.g.* nicotine patches, sprays, or chewing gums) intervention reduced the complication rates in orthopedic surgery[30][39][42]. These results also demonstrated that the impaired bone homeostasis observed in smokers is not associated with nicotine exposure. It is linked to other molecules generated from the combustion of tobacco.

Although the positive effects associated with smoking abstinence are well-proven, many smokers cannot, wish not or fail to quit cigarette smoking[43]. Unfortunately, non-electronic nicotine replacement therapies fail in most smokers due to a lack of the smoking ritual. Therefore, new technologies are based on preserving the smoking ritual while providing less harmful constituents and maintaining the same nicotine levels found in conventional cigarettes. Tobacco heating systems (THS) avoid tobacco combustion at 800°C by only heating tobacco up to 350°C[44]. Interestingly, a study from our group showed that mesenchymal stem cells and human osteoblast exposure to aqueous extract from THS for 21 days showed less impact on cell viability, function, and oxidative stress levels than CS[45]. Additionally, an osteoporotic-like environment is ‘generated’ on a direct co-culture system containing osteoblast/osteoclast exposure to total particulate matter extract from CS in contrast to THS[46].

Besides, we could also demonstrate, that e-cigarette aerosol does not affect bone morphology, structure, and strength compared with CS in a mouse model exposed to these compounds for six months[47].

Although there is *in vitro* evidence of the less harmful effect of electronic nicotine delivery systems on bone cell function and those devices did not negatively influence bone homeostasis in an animal model; still there is no clinical evidence regarding the role of electronic nicotine delivery systems during the fracture healing after orthopedic surgery.

## 2. Methods and Analysis (including design; selection/treatment of subjects; interventional methods; data analysis)

### 2.1. Aim

The study aims to investigate the role of switching from cigarette smoking to THS on the clinical outcome of closed tibia or femur fractures in patients of the Level 1 Trauma center. Validated and standardized assays and medical state will be evaluated in trauma patients' who smoke conventional cigarettes or switch from CS to using THS throughout six months after surgery relative to control. We hypothesize that switching to THS perioperative to an orthopedic surgery improves the

outcomes during tibia or femur fracture healing in smokers' patients due to reduce bone resorption rate consequent to the diminished activity of CS-activated osteoclasts.

## 2.2. Design

The study will be an open-label, three non-parallel groups, single-center clinical study. Patients from a Level 1 Trauma center, Germany diagnosed and treated for closed tibial fracture, closed femoral shaft fracture, or closed distal femoral fracture, including non-smokers and smokers, will be screened for the study. The inclusion and exclusion criteria are summarized in Table 1.

Table 1 Inclusion and exclusion criteria

| Inclusion criteria | Exclusion criteria |
| --- | --- |
| • Closed tibial fracture, closed femoral shaft fracture, or closed distal femoral fracture (according to AO/OTA: 41A2-41C3, 42A-C, 43A-C, 32A-C, 33A2-3, 33B-C) which is surgically treated within 14 days after the trauma at the Level 1 Trauma center, Germany.<br>• Patients > 18 years of age<br><br>Additional inclusion criteria for the smoking and THS groups<br><br>• Smokers with > 10 packyears smoking history<br>• Smoking history > 10 years<br>• Decision not to participate in the free smoking cessation seminars. | • Legal guardian or loss of capacity to consent.<br>• Refusal to participate in the study.<br>• Open fractures or concomitant injuries or complications requiring surgery existing at the time of surgical indication.<br>• Initial surgical treatment of the fracture has occurred *ex-domo*.<br>• No initial surgical treatment within 14 days of sustained trauma.<br>• Using nicotine delivery electronic devices (*e.g.;* E-cigarette) during the observation process after surgery.<br>• Pre-existing autoimmune, immunological, bone or malignant diseases.<br>• Pregnant, breastfeeding and women of childbearing age with existing desire to have children (during the next 6 months).<br>• History of alcohol abuse or drug abuse.<br>• Taking antioxidants approval by the German Federal Institute for Drugs and Medical Devices (BfArM).<br>• Taking drugs with known effects on bone metabolism (according to Institute for medical and pharmaceutical examination issues (IMPP): allosteric CaSR modulators, bisphosphonates, calcium release inhibitors, alkaline earth ions, RANKL inhibitors, calcitriol, cholecalciferol). |

## 2.3. Selection/treatment of subjects

All participants recruited will be advised of the benefits of quitting smoking and the risk of the adverse outcome of smoking cigarettes during fracture healing. If the participant does not want to quit smoking conventional cigarettes, THS will be offered (preferential study design). All smokers will be offered a certified anti-tobacco addiction training session, aimed at trying to convince participants to quit smoking. For those participants who switch to THS, the trainer will introduce them to the correct use and maintenance of the device. Additionally, for those patients who decide to quit smoking or switch to THS, smoking cessation support will be offered (online) during the entire study period by the trainer. The trainers are qualified nurses with a completed 24 hours Smoke Free Training Course certified by the Institute for Therapy Research - IFT.

## 2.4. Interventional Methods

The study will be composed of 3 groups, all lower limb (tibia or femur) fracture orthopedic trauma patients, who will

undergo surgery (internal or external fixation, which involves using screws, plates or nails to hold the bone fracture). The study group will be the following: Smokers willing to switch to THS (experimental), Smokers (no intervention) and Ex - Smokers (active comparator - control) (figure 1).

After the fracture is diagnosed, the pre-operative phase is up to one to two weeks depending on surgery scheduling. During this pre-operative phase, the participant's recruitment, enrollment, smoker's decision to quit smoking or switch to THS, and switching training process will start. The post-operative phase lasts about six months as the expected healing time for tibial or femoral fractures. During this pre-and post-operative phase, five visits will take place at the Level 1 Trauma center Germany according to the standard clinical protocol (figure 1).

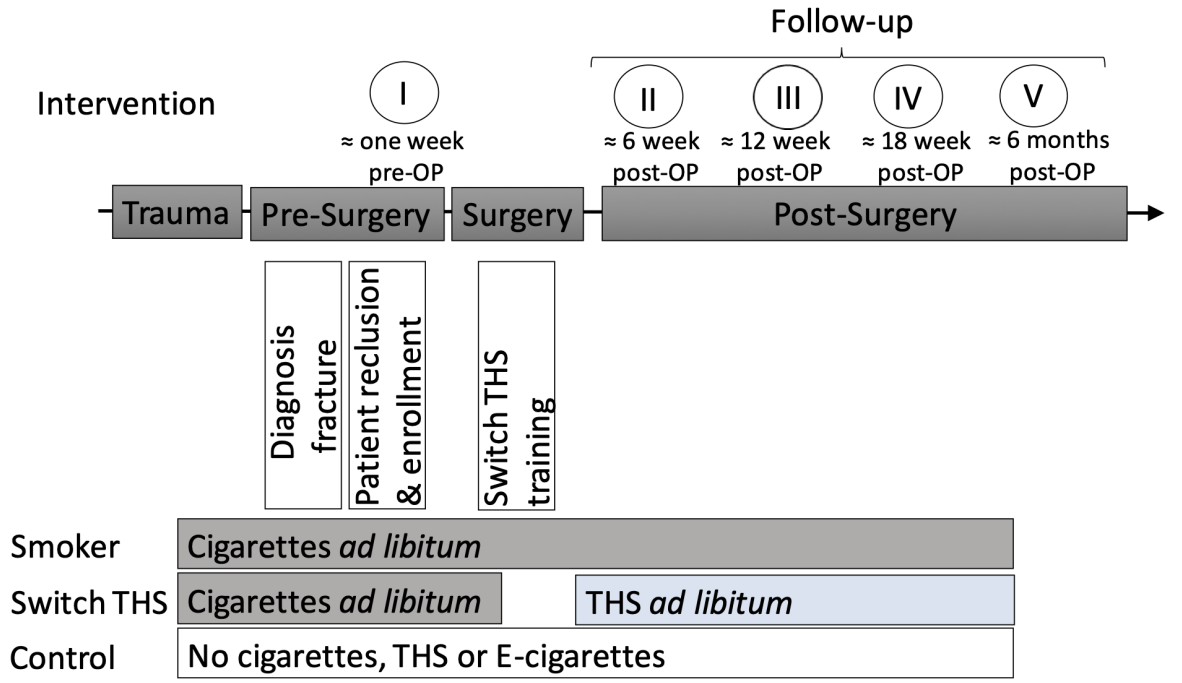


**Figure 1.** Experimental setup.

During the first visit (approximately between 1- or 2 weeks pre-surgery), the participants will be submitted to answer the initial questionnaire. This initial questionnaire will collect general background information regarding socio-status, smoking habits, level of nicotine dependence, and medical history (estimated with the Fagerström Test for Nicotine Dependence (FTND) and Global Health Issues PROMIS® (Short form)). In addition, the first clinical examination will be carried out, including routine blood sampling, X-ray, computed tomography scan (CT) of the fracture, classification of the fracture as well as planning the surgical intervention.

Following surgery, all smokers participating in this study will be offered a training to quit smoking. Those who did not want to quit smoking but decide switching to THS will further receive the assistance of an anti-smoker trainer.

The second visit will take place approximately six weeks (± 2 weeks) post-surgery, including a clinical examination, with blood sampling, and x-ray defined in the routine clinical protocol. The third and fourth visits are also part of the standard

clinical examinations twelve weeks and 18 weeks (± 2 weeks) after surgery, including blood sampling and X-rays. The last intervention is scheduled approximately six months (± 2 months) after the operation; the orthopedic surgeon will evaluate the participant's clinical-functional outcome, as well as the bone healing through X-ray or CT scan and blood sampling.

During the follow-up phase, participants' smoking status will be monitored upon the visits at the Level 1 Trauma center as well as regularly online (twice a week for the first four weeks and then once a week for the following months) by measuring the breath carbon monoxide levels using the Smokerlyzer® piCO™ (CE 2797, Bedfont, England). All participants will also fill out the self-report follow-up questionnaire every three weeks for the following months. The follow-up questionnaire will collect information regarding the smoking history and urges, nicotine withdrawal syndrome as well as, the ability to perform everyday tasks (estimated by Questionnaire of smoking urges (QSU-b), Global Health Issues PROMIS® (Short form), and Lower Extremity Functional Score (LEFS) respectively).

Additionally, when the visits in the Level 1 Trauma center (defined as I, II, III, IV and V) take place, the determination of white blood cell total count, soluble intercellular adhesion molecule-1, and high-density lipoprotein cholesterol level from blood samples will be analyzed to ensure participants' smoking status and to monitor whether or not patients have switched to THS[48].

## 2.5. Data Analysis

The case report form (CRF) will be used as a data collection tool for the study. Electronic CRFs data will be entered at the clinical trial site by authorized clinical staff via an access-controlled, audit-proof, ICH/GCP-compliant, and validated system. The SecuTrial clinical data management system (CDMS) will be used to collect, process, and store study data. Changes in CDMS can be tracked via an audit trail. The correctness of the entries in the CRF will be confirmed by the dated signature of an authorized investigator. The investigator is responsible for ensuring that all sections of the CRF are completed correctly and that the entries can be verified against the source data. The investigator must verify the CRFs by dated signature/electronic signature at specific points during the study and after completion of the CRF. The entered data is subjected to a plausibility check, implemented directly in the CRF, the monitoring, and the medical review. Implausible or missing data is queried and must be explained. The database is locked after completion of data entry, data cleansing, and a final data check. Analog CRFs data will be entered into a database as recorded in the paper-based CRF. Double data entry will be performed to ensure data quality.

As the study's primary outcome, serum levels of the bone formation marker, N-terminal propeptide procollagen type 1 (CICP), will be determined. As a secondary outcome, the following parameters will be measured/monitored: bone turnover and healing, immunological, clinical, complications, and smoking abstinence. An overview of the endpoint and outcomes to be determined in the study is shown in Table 2.

**Table 2.** Summary of parameters and time point for the study

| Outcomes | | Visits | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Initial Questionnaire** | | X | | | | |
| **Classification of the injury** | Injured side | X | | | | |
| | AO classification | X | | | | |
| | Tscherne/Oestern classification | X | | | | |
| **Details of surgical care and aftercare** | Time between accident and first treatment of the fracture [days] | X | | | | |
| | Time between accident and definitive treatment of the fracture [days] | X | | | | |
| | Number of operations | X | | | | |
| | Type of definitive treatment (fixator, plate osteosynthesis, nail osteosynthesis, combination, others) | X | | | | |
| | Load specification (sole contact load; partial weight-bearing; full weight-bearing) | X | | | | |
| **Follow up Questionnaire** * | | X | X | X | X | X |
| **Adverse events** | a- Wound healing disorder (yes/no) | X | X | X | X | X |
| | b- Fracture related Infection (yes/no) | X | X | X | X | X |
| | c- Implant failure | X | X | X | X | X |
| | d- radiological relaxation signs (yes/no) | X | X | X | X | X |
| | e- secondary displacement of the fracture (yes/no) | X | X | X | X | X |
| | f- Thrombosis/embolism (yes/no) | X | X | X | X | X |
| | g- Pneumonia (yes/no) | X | X | X | X | X |
| **Clinical parameters** | Range of motion knee joint Neutral-0 Method: Extension/Flexion: X°/X°/X° | X | X | X | X | X |
| | Ankle Range of Motion Neutral-0 Method: Extension/Flexion: X°/X°/X° | X | X | X | X | X |
| | Achieved limb load: Absolute in N, and as % of body weight | X | X | X | X | X |
| | Function IndeX for Trauma Score | X | X | X | X | X |
| **Bone turnover parameters** | Tartrate-resistant Acid Phosphatase [U/l] | X | X | X | X | X |
| | Bone-specific Alkaline Phosphatase [µg/L] | X | X | X | X | X |
| | Osteoprogesterin [pg/ml] | X | X | X | X | X |
| | Osteopontin [ng/ml] | X | X | X | X | X |
| | N-terminal telopeptide [ng/ml] | X | X | X | X | X |
| | Procollagen Type 1 N-Terminal Propeptide [ng/ml] | X | X | X | X | X |
| **Immunological parameters** | IL-1β [ng/ml] | X | X | X | X | X |
| | IL-6 [ng/ml] | X | X | X | X | X |
| | TNF-α [ng/ml] | X | X | X | X | X |
| | IFN-γ [ng/ml] | X | X | X | X | X |
| **Fracture repair parameters** | RX number cortices bridged | X | X | X | X | X |
| | CT number cortices bridged | X | | | | X |
| | Bone Stiffness [kPa] | X | | | | X |
| **Blood analysis** | leucocytes [N°/µl] | X | X | X | X | X |
| | erythrocytes [mio/µl] | X | X | X | X | X |
| | hemoglobin [g/dl] | X | X | X | X | X |

| | | | | | | |
|---|---|---|---|---|---|---|
| | thrombocytes [N°/µl] | X | X | X | X | X |
| | hematocrit [%] | X | X | X | X | X |
| | Mean corpuscular hemoglobin [pg] | X | X | X | X | X |
| | Mean corpuscular volume [fl] | X | X | X | X | X |
| | corpuscular hemoglobin concentration [g/dl] | X | X | X | X | X |
| | protein c reactive | X | X | X | X | X |
| | white blood cell total count | X | X | X | X | X |
| **Smoking abstinence parameters** | carbon monoxide * | X | X | X | X | X |
| | soluble intercellular adhesion molecule-1 | X | X | X | X | X |
| | high density lipoprotein cholesterol levels (HDL) | X | X | X | X | X |
| **Complication parameters** | Hospital stay [days] | | | | | X |
| | Infections incidence | | | | | X |
| | Wound healing disorder incidence | | | | | X |
| | Further operations incidence | | | | | X |
| | Thrombosis incidence | | | | | X |
| | Duration of incapacity for work [days] | | | | | X |

* *additional online monitoring every 3 weeks,* * * *additional online monitoring twice a week the first 4 weeks, then once a week*

## 2.6. Case number

The number of cases was calculated in consultation with the Institute for Clinical Epidemiology and Applied Biometry at the Eberhard Karls University of Tübingen. If bone cells are exposed to cigarette smoke, osteoblasts activity is significantly reduced, while osteoclasts is increased, leading to osteoporotic changes in the bone[45][46][49][50]. *In vitro* results show that exposure to THS extract does not significantly affect the homeostasis of bone-forming and bone-resorbing cells compared to conventional cigarette smoke[45][46].

We hypothesize that smokers will have significantly lower serum CICP levels than patients using a THS. In contrast, we do not expect a significant difference in serum CICP levels between patients using THS and the control group.

According to our hypothesis, the groups "smokers", "controls" and "THS" will be formed. The primary research hypothesis is that the CICP levels are higher in the THS group than in the smoker group. Furthermore, the known difference between controls and smokers should be confirmed. The comparison between the THS group and the control group is, therefore, exploratory. The empirical basis of the case number estimation is summarized in Table 3.

**Table 3.** Number of cases calculation based on[24]. Depicted are the average concentration of CICP in ng/L and the standard deviation.

| CICP [ng/L] | | | | | |
|---|---|---|---|---|---|
| Smoker | | THS | | control | |
| Average | Standard deviation | Average | Standard deviation | Average | Standard deviation |
| 92.7 | 47.4 | 120 | 32.6 | 127.9 | 29.5 |

The case number estimation was carried out for a one-factorial analysis of variance with different group sizes. The standard deviation was conservatively set at the maximum value of 47.4 for all groups. To demonstrate a difference between the three groups with a significance level of 5% for the overall test in the one-factorial analysis of variance with 80% power, 40 smokers, 40 THS users, and 50 controls are sufficient. For the pairwise comparison between THS users and smokers, a power of 83% results, and for the pairwise comparison between controls and smokers, a power of 97%, both with two-sided testing at the 5% significance level. Due to the study's cross-sectional nature, a very low drop-out rate is expected; should drop-outs occur, they will be recruited. The calculations were carried out with nQuery release 4.0. The analysis will be carried out in two stages, initially as an overall test to compare all three study groups in a one-way analysis of variance. If no differences are found, the test procedure is terminated. If the overall test is significant, all three pairwise comparisons will be carried out without correction for multiple testing. The primary evaluation population is the modified intention to treat population, which consists of all participants with a primary endpoint of CIPC (6 months follow-up). Interim and subpopulation analyses as well as imputation of missing values are not planned.

## 3. Discussion

According to the German Ministry of Food and Agriculture, CS causes 25.4 billion euros in direct costs for the social security system every year, of which 22.76 billion euros are spent on medical treatment[51]. In the United States, the National Center for Chronic Disease Prevention and Health Promotion reported more than 240 billion dollars cost associated with CS are spent in healthcare. In addition to the harmful health aspects, socio-economic reasons highlight the need to reduce cigarette smoking prevalence. Cigarette smoking has been shown to lead to an increased risk of bone fracture[11][14], delayed fracture healing[52], failure of healing[9], and an increased rate of postoperative complications[21], resulting in prolonged hospitalization[9][22][23][24][53]. Complications, in particular, , cause especially high costs as they are often associated with intensive care stays, revision operations, or interventions of all kinds[54]. Our previous retrospective study demonstrated that current and former smokers had a significantly more extended hospital stay of 18.4 days compared to non-smokers, who were discharged after 15.3 days on average[24]. The immobilization associated with the longer hospital stay increases the risk of other adverse events, such as thrombosis. This results in an additional burden for the healthcare system and society[54].

So far, cigarette smoking cessation is the only alternative proven to reduce harmful effects on the human body[21]. Several studies have shown a reduced postoperative complication rate for patients who quit smoking cigarettes preoperatively[40][41], whereby the World Health Organization suggested four weeks of smoking abstinence prior to surgical intervention[55]. Despite all the positive effects associated with smoking cessation, many smokers are unable or

unwilling to quit cigarette smoking or fail in their attempts. Without additional support alternatives or therapy, an attempt to quit smoking after one year is successful on average in only 3-5 % of cases[56]. For instance, the retrospective study from Hall *et al.* showed that only 23% of total joint arthroplasty patients were able to quit smoking for one year[57].

Although there are many nicotine-based replacement alternatives on the market, such as gum, patches, and sprays, the lack of ritual provides a major disadvantage that minimizes the chances of success in quitting cigarette smoking[58]. Therefore, it is essential to explore alternatives that support patients to quit smoking but maintain the ritual associated with CS.

THS are newly developed technologies to reduce the consumer's exposure to potentially harmful substances produced during tobacco combustion, as well as maintain smoking rituals and provide similar nicotine levels to cigarettes[59][60]. Given this, it can be assumed a high level of acceptance by smokers.

*In vitro*, a significantly less harmful effect of THS compared to CS on mesenchymal stem cells and human osteoblast has been demonstrated[45]. Additionally, an osteoporotic-like environment was generated on a direct co-culture system containing osteoblast/osteoclast exposure to extract from CS in contrast with THS[46]. The described *in vitro* results suggest that THS may be a less harmful alternative for smokers' orthopedic patients concerning fracture healing. However, the effect of switching from cigarettes to THS on the fracture healing process has not been explored in humans. Therefore, this study tests the hypothesis that switching to a THS after prior orthopedic surgery improves outcomes in orthopedic smoking patients during lower limb fracture healing over six months.

The main strength of this prospective, open-label study will be evidence of increased serum concentration of CICP (primary outcome) in THS participants compared to smokers due to a reduced bone resorption rate. The study will also examine additional secondary parameters related to bone metabolism, bone healing, immunological, blood count, and clinical and sociodemographic parameters that facilitate our understanding of the overall status of the participants.

There are potential limitations to the study that need to be acknowledged. This study explores the effect of the switch from CS to THS only on fracture healing of lower limb "long" bones. Since maxillofacial bones are directly exposed to the particulates contained in smoke or aerosols generated by cigarettes or THS molecules, the influence on bone cells homeostasis may differ from long bones. Additionally, blood sampling will take place during the clinical interventions in the late morning (between 9 and 12 PM). However, serum CIPC concentrations have a circadian variation, with the highest concentration detected in the early morning[61], potentially causing the differences between the groups to be less significant.

## Notes

**Trial registration:** The study is registered on ClinicalTrials.gov (NCT05859451).

## Abbreviations

- BfArM: German Federal Institute for Drugs and Medical Devices
- CDMS: Clinical data management system
- CICP: N-terminal propeptide procollagen type 1
- CRF: Case report form
- CS: Cigarette smoke
- CT: Computed tomography scan
- ENDS: Electronic nicotine delivery systems
- FTND: Fagerström Test for Nicotine Dependence
- GCP: Good clinical practice
- ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
- IFT: Institute for Therapy Research
- IMPP: Institute for medical and pharmaceutical examination issues
- LEFS: Lower Extremity Functional Score
- QSU-b: Questionnaire of smoking urges
- THS: Tobacco heating system
- ZKS: Center for Clinical Studies

## Statements and Declarations

### Conflicts of Interest

The study was partially funded by Philip Morris International. However, the authors declare no personal conflicts of interest related to this work.

### Ethics Statement

The study protocol and the declarations of consent were approved by the ethics committee of the medical faculty of Eberhard Karls University (724/2022BO1). Written informed consent to participate in this study will be obtained from participants.

### Monitoring procedure

Monitoring for this study is provided by the Zentrum für Klinische Studien Tübingen (ZKS Tübingen). The monitoring will be conducted according to ZKS Tübingen internal Standard Operating Procedures (SOPs) and a dedicated monitoring manual for the study. The monitoring timelines include initiation visit, regular monitor visits during the course of the trial as well as a close out visit. Usually, Monitoring will end with the last visit after full documentation of the last patient enrolled (close out visit). All investigators agree that the monitors regularly visit the trial site, assure that the monitors will receive

appropriate support in their activities and will have access to all trial-related documents. The aim of the monitoring is to ensure patient safety and rights, data accuracy, and that the study is conducted in accordance with the approved protocol and applicable regulations.

### Data Availability

Documents required to support the study protocol can be supplied on reasonable request to the corresponding author. After study closure, anonymized data will be made available upon reasonable request to the corresponding author.

### Author Contributions

The study was designed by RA, MH, BB and AKN. RA and MH wrote the original draft of the manuscript. Besides, AKN served as the project administrator. All authors provided critical feedback and reviewed, edited, and approved the final manuscript.

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