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Application of a Novel Bone Osteotomy Plate Leads to Reduction in Heat-Induced Bone Tissue Necrosis in Sheep

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ABSTRACT

Previous studies have shown substantial effect thermal damage can have on new bone formation following osteotomy. In this study we evaluated the extent of thermal damage which occurs in four different methods of osteotomy and the effects it can have on bone healing. We further wanted to test whether a special osteotomy plate we constructed can lead to diminished heat generation during osteotomy and enhanced bone healing. The four methods evaluated included osteotomy performed by chisel, a newly constructed osteotomy plate, Gigly and oscillating saw. Twelve adult sheep underwent osteotomy performed on both tibiae. Bone fragments were stabilized using a fixation plate. Callus size was assessed using standard radiographs. Densitometry and histological evaluation were performed at 8 weeks following osteotomy. Temperature measurements were performed both in vivo during the operation, and ex vivo on explanted tibiae. The defects healed without complications and showed typical course of secondary fracture healing with callus ingrowth into the osteotomy gap. Radiographic examination of bone healing showed a tendency towards more callus formation in bones osteotomized using Gigly and oscillating saw, but this difference lacked significance. Use of Gigly and oscillating saw elicited much higher temperatures at the bone cortex surface, which subsequently lead to slightly impaired bone healing according to histological analysis. BMD was equal among all bones. In conclusion, the time required for complete healing of the defect differed depended greatly on the instruments used. The newly constructed osteotomy plate showed best results based on histological findings of capillary and osteoblast density.

Key words: osteotomy, thermal damage, new-constructed plate

Introduction

Mechanical conditions of osteotomy significantly affect the biological process of bone healing. Previous studies have identified numerous mechanical parameters such as gap size, strain rate and magnitude, the nature of loading, the timing of mechanical stimuli during healing and even muscle activity that affect the healing process. Many additional factors influence fracture repair, among them; the severity of injury, type of fracture, vascular damage, method of treatment, infection, age of patient, hormonal and nutritional factors and systemic disease.

Secondary fracture healing is known to be accelerated by the process of periosteal callus formation¹. Osteoblasts are cells largely responsible for the formation of fracture callus².

During the osteotomy as well as bone reaming, significant amount of heat is produced. The generation of heat is one of the most serious problems encountered when bone is cut or drilled. A number of studies describing the deleterious effects of elevated temperatures on bone have

been published thus far³⁻⁵. Bone temperature above 47 °C causes irreversible bone necrosis, with bone resorption at cutting (or drilling) site⁶. Different degrees of damage can occur based on thermal damage. Thermal damage influences vascular and cellular response during bone healing.

Although different approaches have been used so far, such as biological, radiological or histological, to investigate all the aspects of heat-impaired bone healing, at present a lot still remains to be elucidated regarding the effects of thermal damage at the osteotomy site. Therefore we experimentally investigated in sheep tibia how the heat generated by different osteotomy methods affected bone union. Radiographic x-ray, BMD and histological findings revealed significant impact the heat generated by osteotomy can have on new bone formation. Furthermore, we also investigated whether the use of a newly constructed osteotomy plate can alleviate thermal damage and lead to enhanced rebridgement of the osteotomy.

Materials and Methods

Animals

Twelve adult Pramenka female sheep weighing 36.8 ± 1.6 kg (mean \pm SD) were used. The sheep were fed with

standard animal food. Access to tap water was free. Temperature in the cages was 22 °C and humidity 60°. All animal experimentation was reviewed and approved by the Animal Committee of Ministry of Agriculture and Forestry.

Operating procedure

The sheep were left without food for a 24 h before a surgery procedure, and water 6 hours before. Xilazin (Xhylapan, Chassot) was used for premedication in dose 0,1 mg/kg IM. After 20 minutes intravenous catheter (20G) was introduced in v. cephalica antebrachii. After this the Ketamine (Narketan, Chassot) was applied in dose 6 mg/kg IV. Using laryngoscope the animals were intubated endotracheally using endotracheal tubus interior diameter 10 mm. After this animals inhaled Oxygen (10–20 mL/kg/min) and Halothane (Fluothane, Zeneca)(first few minutes 3%, and then 2% during 90 minutes) mixture. Epidural analgesia using Lidokain 2% (Lidokain 2%, Belupo) in dose 1 mL/5kg body weight was performed.

During the surgical procedure the animals applied Ringer solution flowing 10 mL/kg/h IV Just before, and as well after the surgical procedure prophylactic the Cefuroxim (Ketocef, Pliva) was applied in dose 22 mg/kg IV.

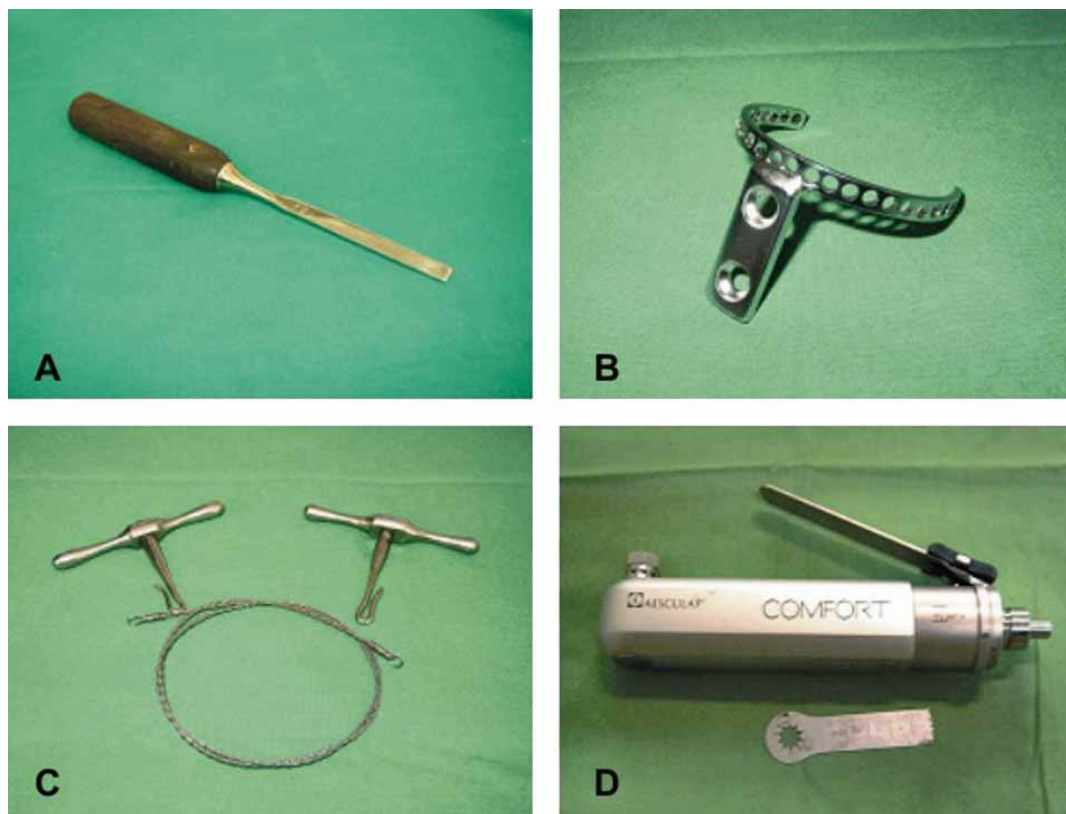


Fig. 1. Osteotomy instruments. Instruments used in the study included: chisel (a), special newly designed osteotomy plate (b), Gigly saw (c) and Oscillating saw (d).

Seven days after the surgically procedure antibiotic Enrofloxacin (Vetoflok 10%, Veterina) in dose 10mg/kg IM was applied. Cefuroksim (Ketocef, Pliva) 22 mL/kg introduced intravenously was performed 7 days after the operation.

A longitudinal incision with medial approach was made on the both tibiae. Periosteal tissue was incised and elevated. A transverse osteotomy was performed in the mid-diaphysis with four different instruments and then fixed using a conventional 3.5 mm DC Plate with six fixating screws. The four osteotomy methods used included chisel, a newly constructed plate, Gigly and oscillating saw (Figure 1). The animals were assigned to different experimental groups at random. During the operation a thermocouple (Control company) was used to monitor the osteotomy induced temperature. The sterile electrode was placed 5 mm proximally from the osteotomy gap. We used irrigation with 0.9 % NaCl during osteotomy.

The animals were administered post-operative analgesia for a period of 5 days. The sheep were allowed full

weight bearing immediately following the operation without immobilization. The wound was dressed properly every day, using sterile wound closure. After ten days the sutures were removed. Only one postoperative complication was observed: in the first operated animal, refracture happened followed by infection and sepsis five days following the operation. The animal exited in spite of the applied antibiotic therapy.

Radiographic analysis and DXA measurement

Radiographs were taken in the standard antero-posterior and lateral projection (Polydos, Siemens, Germany, 42 kV, 10 mAs, 1 m) immediately after surgery, three and eight weeks following operation. After the animals were sacrificed 8 weeks following the operation, anteroposterior and lateral radiographs and bone density of the osteotomized tibiae were performed. The right tibia was disarticulated at the knee joint, carefully dissected with the periosteum and callus formation intact, and the plate and screws were extracted. After the tibia bones were re-

TABLE 1
RADIOGRAPHIC CALLUS CHARACTERISTICS IN OSTEOTOMIZED BONES 8 WEEKS FOLLOWING SURGERY

Radiographic analysis of the callus formation				
Animal No.	Thickness (cm)	Length (cm)	Form	Fracture gap
Chisel				
1	1	8	linear	+
2	0.7	9	linear	-
3	0.6	4	linear	-
4	0.4	7	linear	-
5	1	8	linear	+/-
6	0.5	6	linear	-
Newly constructed plate				
7	0.8	8	linear	-
8	0.5	8	linear	-
9	0.5	6	linear	+/-
10	1.1	11	linear	-
11	0.5	5	linear	+/-
12	0.8	8	linear	-
Gigly saw				
13	1	8	linear	+
14	0.9	7	unequal	+
15	0.5	8	unequal	+
16	0.5	8	unequal	+
17	0.9	7	unequal	+
Oscillating saw				
18	0.9	8	linear	+
19	1	5	linear	+
20	1	10	linear	+
21	0.7	5	linear	+
22	0.9	8	linear	+

moved and fixed in 70% ethanol, bone mineral density (BMD mg/mm³) and bone mineral content (BMC mg/mm) measurement by DEXA method was performed using dual-energy X-ray absorptiometry (DXA; Hologic QDR-4000, Hologic, Waltham, MA, USA), equipped with a Regional High Resolution Scan software. The scan field size was 5.08 × 1.902 cm, resolution was 0.0254 × 0.0127 cm, and speed was 7.25 mm/s. The scan images were analyzed and the bone area, bone mineral content and bone density of whole bones, proximal and distal metaphyses and the shaft of tibiae were determined.

Histological analysis

The medial section of the osteotomized area was designated for the histological evaluation. The tibiae were fixed in buffered 10% formalin, dehydrated in graded alcohols, defatted in xylene, and embedded in methyl-methacrylate using standard methods, and 100 μm sections were cut with a milling bone saw (Leitz 1600, Germany). Slices in the longitudinal plane were cut in the middle of the tibial shaft and the callus. The histological sections were evaluated microscopically (light microscope, Diaplan,) and imaged with a color ccd camera (Monochip color RGB TK 1270, JVC, Japan) using a ×25 objective. The callus was analyzed qualitatively.

Ex vivo thermographic analysis

Thermal changes elicited during osteotomy were recorded *in vivo* during surgical procedure using thermocouple and *ex vivo* using explanted sheep tibiae. The samples, recorded with thermographic camera during osteotomy on isolated bones were analysed using Ther-

maCAM Explorer 99 (publish 2003) PRODUCER flir Systems (Figure 7). The effect of irrigation was also evaluated during osteotomy.

Statistical methods

Frequencies and standard descriptive statistics were used to describe the study groups. Means, standard deviations (SD), minimal and maximal values were calculated for continuous variables, while frequency tables were tabulated for categorical variables. Continuous variables were compared using ANOVA. Duncan's multiple range test was used as the post hoc test. The chi-squared test was used to compare categorical variables. Differences between groups were considered to be significant for variables yielding $p \leq 0.05$. All tabulations and statistical analysis were done using Statistica for Windows, Version 5.5, StatSoft, Inc. (2000).

Results

Radiological findings

RTG was performed immediately following the operation, as well as at three and eight weeks. No external callus was detectable on radiographs at 3 weeks after the bone fracture. With time, the callus appeared to surround the osteotomized site. Full rebridgement of the defect was detectable in all animals at eight weeks following osteotomy. The callus size was much more prominent in the bones which were osteotomized using Gigly and oscillating saw (Figure 2 C, D), but there were no statistically significant differences in the thickness and length

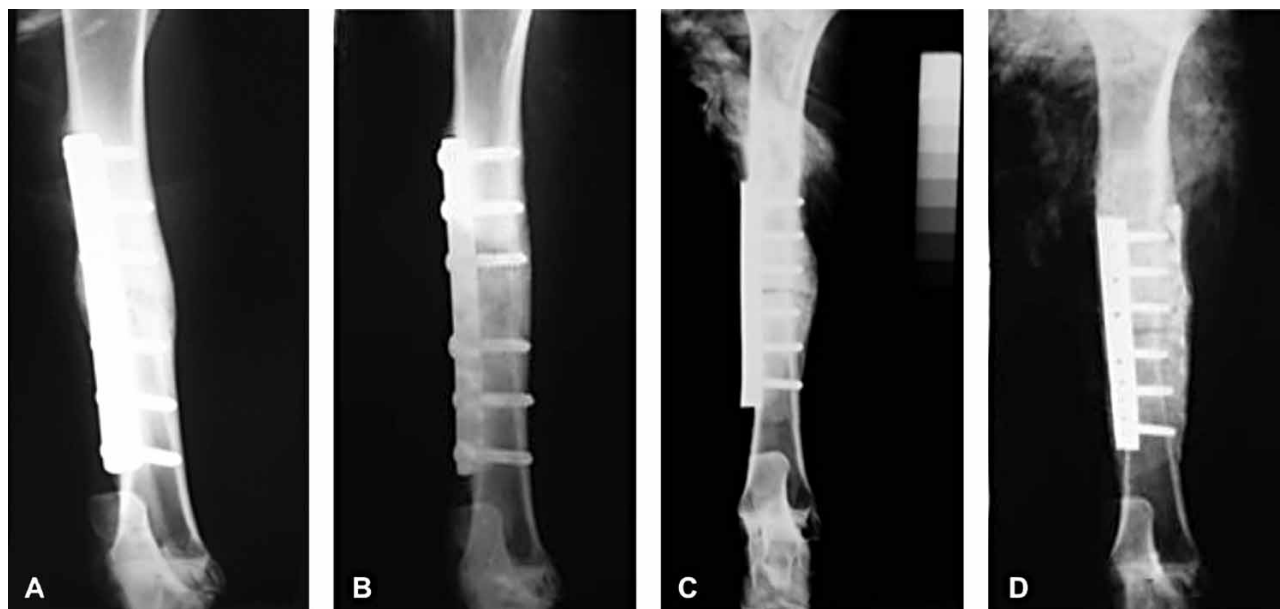


Fig. 2. Radiographic assessment of healing. Lateral radiographs of representative tibia specimens 8 weeks following osteotomy. Tibiae osteotomized using chisel (a) and the newly constructed osteotomy plate (b) exhibit fracture gap fully filled with new bone callus and with linear periosteal callus and primary fracture gap not visible. Radiographs of tibia specimen osteotomized using a Gigly saw (c) exhibit a visible fracture gap and an uneven and abundant periosteal callus. The use of oscillating saw (d) led to uneven callus formation and incomplete rebridgement of the defect.

formation at eight weeks following osteotomy ($p > 0.05$) (Figure 3 A, B). Fracture line and screw holes were no longer visible in tibiae osteotomized with the newly constructed plate or chisel at the same time-point (Figure 2 A, B). On the other hand, osteotomy gap and screw holes were visible even after eight weeks in bones osteotomized with Gigly and oscillating saw (Table 1).

BMD Bone mineral density measurement

Densitometric examination was performed *ex vivo* on explanted tibiae, with soft issue carefully stripped and screws and plates removed. The results in the BMD analysis among four groups of bones were similar and there was no statistically significance among these osteotomized bone eight weeks postoperatively (Figure 3 c, d).

Histological findings

All osteotomies showed typical signs of secondary fracture healing: callus formation starting approximately between 6 mm and 8 mm away from the osteotomy line; the front of cartilage calcification reaching the osteotomy line; total bridging of the periosteal callus by bone.

Osteoblastic cell proliferation was significantly greater in bones osteotomized using the newly constructed plate, while bones osteotomized with chisel exhibited the lowest osteoblastic density (Figure 4a). The capillary density was significantly greater among bone specimens osteotomized using chisel ($p < 0.05$). The lowest capillary density in callus formation was observed among specimens osteotomized using the Gigly saw ($p < 0.05$) (Figure 4B). In some osteotomized bone species at the end of the 8 week postoperative period, the gap was completely filled with bone, and these bones reached the phase of remodeling. Histological examination revealed enchondral new bone formation in all four groups. (Figure 4 c, d and e).

Temperature measurement

The temperature increase at osteotomy site was detected during surgical operation *in vivo* and *ex vivo*, on explanted bone samples. The highest increase in bone temperature at the osteotomy site was measured in bones osteotomized using Gigly saw with the median value of 71.8 ± 0.59 °C. This was statistically significant temperature increase ($p < 0.05$). The mean bone temperature

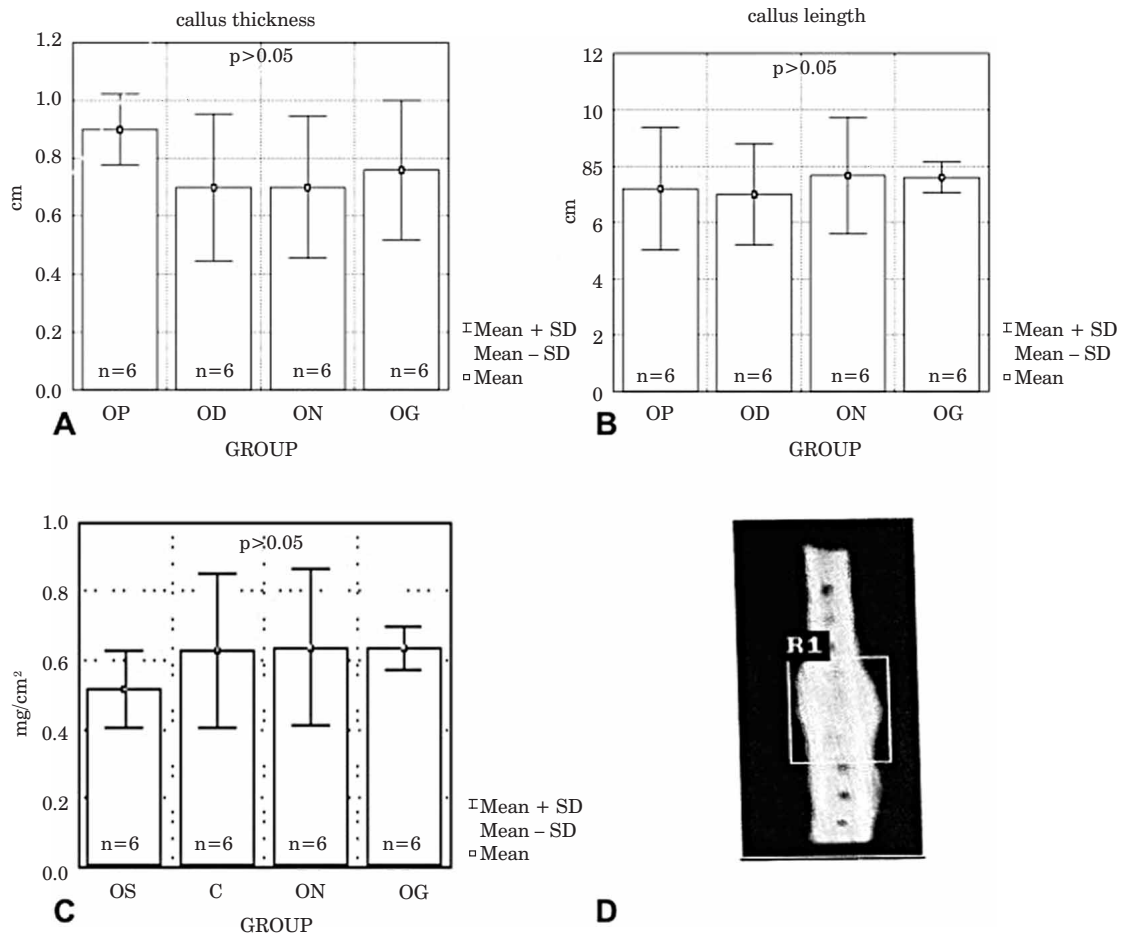
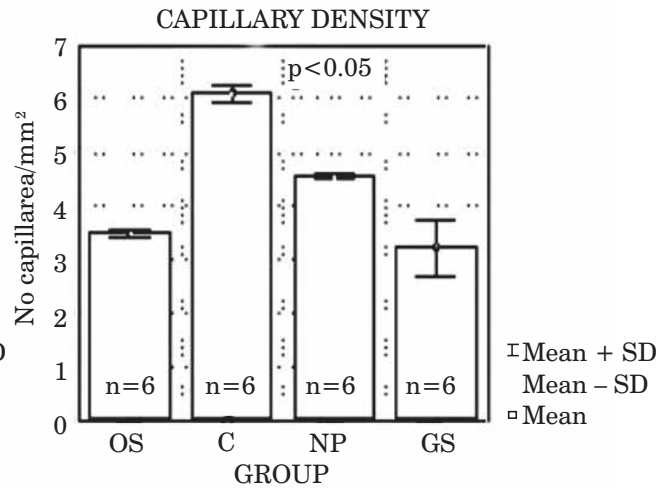
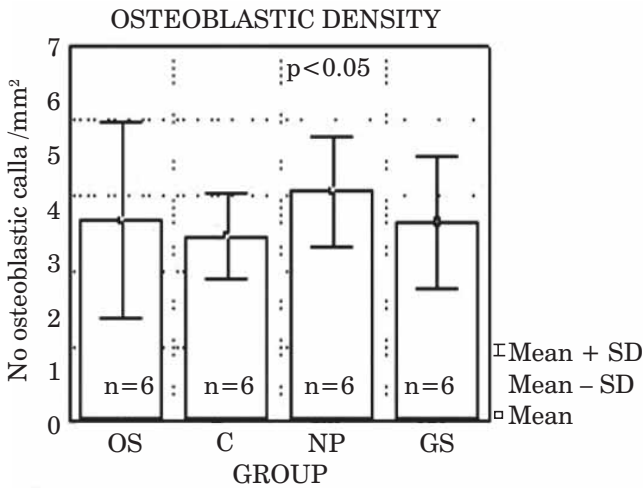


Fig. 3. Radiographic and densitometric properties of the newly formed callus. Callus thickness (a) and length (b) measured on radiographs 8 weeks following osteotomy. Densitometric view (c) and bone mineral density (d) of the newly formed callus 8 weeks following osteotomy. R1 – area of interest

value recorded in bones osteotomized using the newly constructed plate was 45.9 ± 0.58 °C, while the use of oscillating saw elicited 43.3 ± 0.32 °C. The temperature changes were not recorded at all in bones osteotomized using a chisel. There was no difference among tempera-

ture values measured in bones *in vivo* and *ex vivo*. All temperature values mentioned above were detected without using irrigation. Use of irrigation with 0.9 % NaCl during osteotomy, lead to all measurements showing temperature values within physiological borders (Figure 5).



A

B

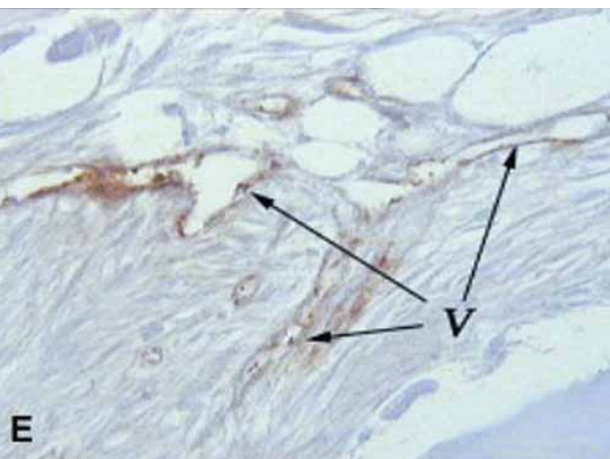
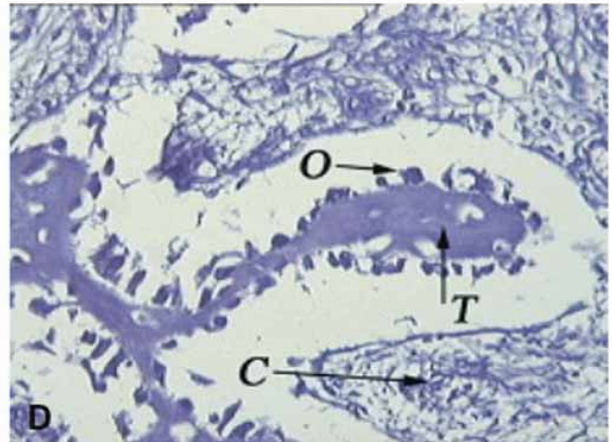
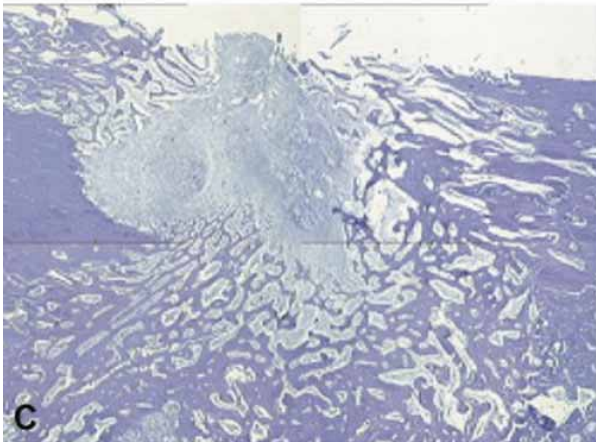


Fig. 4. Histological analysis of newly formed bone. Osteoblastic density (a) and capillary density (b) of the calluses was assessed. Histological longitudinal sections through the metatarsal (c) exhibited partial periosteal and endosteal bridging of the osteotomy gap with predominant fibrocartilagenous and connective tissue formation. Sections through the callus tissue (d) showed bony trabeculae (T) with many osteoblasts (O) and connective tissue (C). The newly formed callus also exhibited increased neo-angiogenesis with blood vessel (V) distribution (E) (immunohistochemistry, von Willenbrandt factor vWF). All histological images were taken with $\times 25$ objective.

Discussion

This study attempted to investigate the best technique of osteotomy in fracture healing process. The newly constructed osteotomy plate is specially designed to provide precise osteotomy with minimal heating of the bone and surrounding tissue. The use of different instruments (newly constructed osteotomy plate, chisel, Gigly and oscillating saw) showed different levels of thermal damage. In our experiment we tried to achieve the conditions similar to those we meet in the nature and clinical practice.

It is well accepted that inter-fragmentary movement influences the fracture healing process. Small axial movements can stimulate callus formation whereas larger shear movement delays the healing process^{7,8}. Small callus formation is achieved with a generally stable fixation where as a larger callus forms with an unstable fractures fixation^{9,10}. Therefore, to avoid larger interfragmentary movement and to secure early postoperatively sheep movement we used rigid internal fixation with DC plate and osteotomy 1–2 mm gap.

To our knowledge there are no published studies dealing with correlation between the method of osteotomy and thermal bone damage. Therefore, this is the first study to indicate the effect of different types of instruments used for osteotomy on thermal damage and bone healing.

During the fracture healing cells proliferate, differentiate and blood vessels grow into the new formed callus. These processes are influenced by different growth factors, cytokines and growth hormones. Cell proliferation is a very fundamental and early event during fracture healing. Also, recruitment of a blood supply is critical for successful bone induction and fracture healing^{2,11}. The morphology of the callus, the angiogenesis and the proliferation of osteoblastic cells showed differences in the four groups of bone samples after osteotomy during healing process.

Thermal damage to tissue is related to the maximum temperatures to which the tissues are exposed, and to the length of time that the tissues are subjected to the damaging temperatures. Bone heated to more than 45 °C leads to osteocyte necrosis, deactivation of alkaline phos-

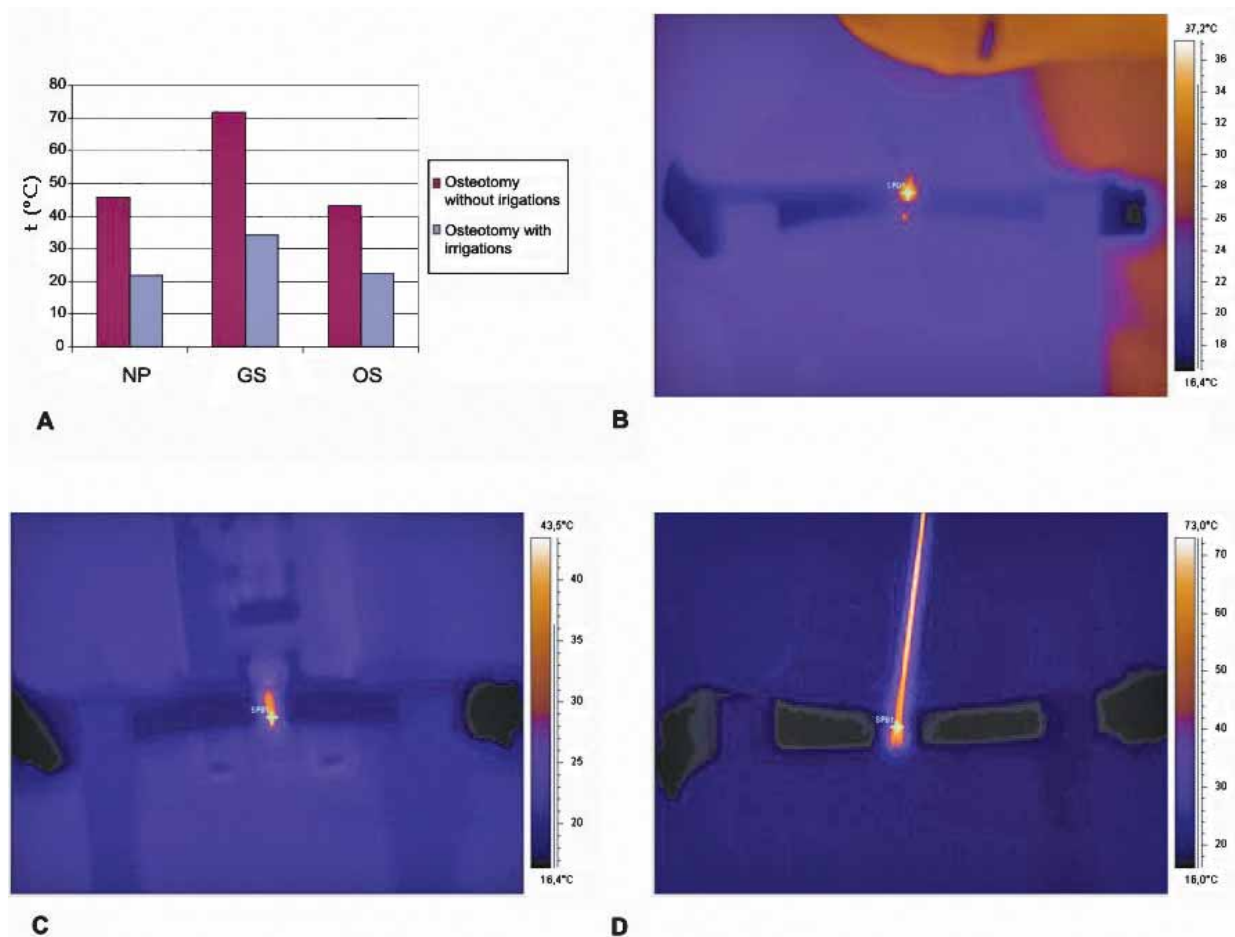


Fig. 5. Temperature measurements during osteotomy. Temperature measured during osteotomy (a) using the newly designed plate (NP), Gigly (GS) and oscillating saw (OS). Thermographic images of the osteotomy procedure using the newly designed plate (b), Gigly (c) and oscillating saw (d).

phatase and degradation of collagen-hydroxyapatite bone. This results in permanent alteration in the mechanical properties. Bonfield and Li¹² found that the mechanical properties of bone were irreversibly altered by heating to 50 °C. A temperature of 53 °C below the denaturation point of alkaline phosphatase caused an irreversible bone injury, after which healing occurred from the surrounding tissues¹³. Temperature of 56 °C at which alkaline phosphatase is denaturated is critical¹⁴. Bergman¹⁵ found at the temperature of 70 °C first histological evidence of thermal bone necrosis showing osteon cellular loss and disruption of the orderly lamellar bone matrix. Matthews and Hirsch³ have measured substantially lower drilling temperatures when irrigating by directing a stream of water where the drill penetrates the bone.

The extraosseous soft-tissue envelope is the predominant source of revascularization to a diaphyseal fracture. Vascular invasion is a crucial step in osteogenesis that usually occurs in models of matrix-induced endochondral ossification¹⁶. The regenerating bone capillaries may provide a source of osteogenic cells^{11,17}.

BMD is often used in experiments to show the risk of fracture. Bone density still represents the primary determinant of bone strength^{18–20} and correlates with the biomechanical status of healing callus at least in the early phase of fracture healing²¹, especially when combined with geometric bone properties^{22,23} also some investigators⁹ maintained unreliability of bone density as only parameter to estimate the strength of healing bone.

Huge callus volumes that are not bridged at the fracture line indicate an appropriate external loading. The amount of periosteal callus therefore, is not by itself a suitable predictor of the rigidity of the fracture. Increased periosteal callus formation is rather caused by insufficient mechanical stability and is therefore not an indicator of a successful healing process^{23,7}. Noninvasive quantitative bone measurements can be helpful in estimate the mechanical stability of a healing long bone. Histological information proved to be the best predictor of mechanical stability⁷.

A new bone area in week 8 was clearly visible in all animals but there was no significant difference in callus size between four groups. Histologically, secondary fracture healing occurred in all ship by histological analysis, the callus size periosteal reaction, vascularity and cellularity in the treated osteotomies we suggest that the use of new constructed osteotomy plate may provide a significant advance in the treatment of fracture. New constructed plate can stimulate bone healing minimizing the thermal and mechanic damage. So we can see the importance to the kind of instrument to avoid the heat generated by bone saws when osteotomy is done. An understanding of the cellular and molecular pathways that govern the events of fracture healing is critical to the advancement of fracture treatment. Healing was delayed in those bones osteotomized with Gigly and oscillating saw. Only the bones osteotomized with the newly constructed plate showed evidence of bone regeneration with both, angiogenic and osteoblastic potential.

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UČINKOVITOST UPORABE NOVOKONSTRUIRANE PLOČICE ZA OSTEOTOMIJU NA SMANJENU, TOPLINOM UZROKOVANU, NEKROZU KOŠTANOG TKIVA KOD OVACA

S A Ž E T A K

Iz dostupne literature jasno je vidljiv negativan učinak topline na formiranje novog koštanog tkiva nakon osteotomije. Naše istraživanje je ispitivalo različiti stupanj toplinskog oštećenja kosti primjenom četiri različite tehnike osteotomije. Svrha nam je bila pokazati smanjuje li novokonstruirana pločica za osteotomiju toplinsko oštećenje, čime poboljšava cijeljenje kosti. Vrste instrumenata za osteotomiju koje smo istraživali su bili dljetto, novokonstruirana pločica za osteotomiju, Giglijeva i oscilacijska pila. Kod dvanaest zrelih ovaca smo osteotomirali obje stražnje tibije. Primjenom pločice za osteosintezu smo fiksirali koštane ulomke. Stvaranje kalusa smo pratili na kontrolnim RTG slikama. Osam tjedana poslije osteotomije učinili smo denzitometrijsko i histološku obradu osteotomiranih kosti. Temperaturu koja se oslobađa pri osteotomiji smo mjerili *in vivo* za vrijeme zahvata te *in vitro* na izoliranim kostima. Koštani defekti su cijelili bez komplikacija uz karakterističnu sliku sekundarnog cijeljenja kosti uz urastanje kalusa u osteotomijsku pukotinu. Radiološko istraživanje koštanog cijeljenja je pokazalo pojačano stvaranje kalusa u kostima osteotomiranim Giglijevom i oscilacijskom pilom, ali ova različitost nije bila statistički značajna. Giglijeva i oscilacijska pila oslobađaju znatno veću količinu topline na površini kosti, što rezultira oslabljenim koštanim cijeljenjem što smo pokazali histološkim istraživanjem. Sve kosti su imale sličnu mineralnu gustoću. Zaključno, vrijeme potrebno za kompletno cijeljenje koštanog defekta u velikoj mjeri ovisi o instrumentu koji koristimo u svrhu osteotomije. Na temelju histološkog mjerenja gustoće osteoblasta i kapilara, novokonstruirana pločica je pokazala najuspješnije cijeljenje uz minimalno termičko i biomehaničko oštećenje kosti.