Suitability of Permanent Probe Implants For the Measurement of Intramedullary Perfusion and Temperature Near the Bone Cortex: A Pilot Study Using a Rabbit Model

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Summary

This study was conducted to test the suitability of permanent probe implants for the measurement of intramedullary perfusion by laser Doppler flowmetry and for the measurement of temperature near the bone cortex. Measurements were carried out on the conscious animal in order to rule out the influence of anaesthesia on intramedullary perfusion and temperature. During the first experimental animal trials, some of the probes made of polysulphon broke and/or gave false temperature measurements, so the original probe design was modified. The probes were reinforced with metal, and the temperature sensors were made less permeable to moisture. These modified probes were found to be suitable for permanent measurement of intramedullary perfusion and of temperature near the cortex in the conscious rabbit.

Introduction

Healing of fractures is known to be related to the bone blood flow. This has been demonstrated with the tracer microsphere method (*Grundnes and Reikerås, 1992*) and with the wash-out technique (*Paradis and Kelly, 1975*). However, these techniques are very invasive and require removal or destruction of tissue.

Another recognised, less invasive method for the dynamic measurement of blood flow is laser Doppler flowmetry (*Herzog et al., 2002; Jain et al., 1996 and 2000; Kregor et al., 1995; Lausten et al., 1993; Salerud and Hellem, 1992*). Although only relative blood flow values (Perfusion) can be obtained with laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI), these values have been shown to correspond to those for bone blood circulation obtained with the tracer microsphere method (*Lausten et al., 1993; Shymkiw et al., 2001*).

Previous studies using LDF or LDI to measure cortical or intramedullary perfusion in rabbits were conducted on the anaesthetised animal and without permanent probe implants, in some cases after surgical exposure of the bone (*Chan et al., 1999; Herzog et al., 2002; McDonald and Pitt Ford, 1993 and 1994; Salerud and Hellem, 1992; Shymkiw et al., 2001; Wolf et al. 2000*). However, Jain and coworkers (*1996 and 2000*) described the experimental use of permanent probe implants in the dog for the repeated measurement of perfusion in the tibia during fracture healing after plate osteosynthesis. These investigators compared custom made single-fibre probe implants made of polymethylmethacrylate (PMMA) and coated with a silastic elastomer to conventional probes. For measurement with the standard probes, it was necessary to anaesthetise the animals and to expose the plated bone surgically, but only light sedation was required for the weekly measurements using the single fibre probe implants (*Jain et al., 1996 and 2000*).

There have been no reports of the use of permanent

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probe implants for daily measurement of intramedullary perfusion in the unsedated animal. However, such a model would be advantageous for continual observation of intramedullary perfusion during fracture healing, particularly for the development of resorbable intramedullary implants for the surgical treatment of fractures. Furthermore, there are no publications on the measurement of temperature near the cortex.

It was thus the objective of this study to determine the suitability and functionality of probes designed as permanent implants for the measurement of intramedullary perfusion and temperature near the bone cortex in the conscious animal over a period of 49 days.

Materials and Methods

Animals

Five adult New Zealand White Rabbits with a body weight between 4.0 and 5.0 kg were used in this experiment. The animals were supplied by Charles River, Kisslegg, Germany. The experimental design and use of animals were approved by the responsible agency (Lower Saxony State Authority for Consumer Protection and Food Safety, permit 03/689). The animals were kept in single cages (EC3 rabbit cage system, Scanbur BK, Karlslunde, Denmark) under standardised conditions in compliance with the recommendations of the European Commission (ETS 123, 2003). A twelve-twelve-hour day-night cycle was maintained with continuous monitoring of humidity and air temperature. The rabbits received a daily ration of 150 g complete rabbit diet (K-H, Ered, 4 mm, Sniff Spezialdiäten GmbH, Soest, Germany) and water *ad libitum*. Microbiological status was conventional and pathogen free. The animals were given two months to become accustomed to being handled daily by their animal keepers before the beginning of the experiment.

Experimental design

Measurements of intramedullary perfusion and temperature near the cortex were carried out at three different sites on the tibia of the rabbits for a period of 49 days. The probes for perfusion and temperature measurement were custom-made for the special demands of the experiment by Perimed (Järfälla, Sweden). The body of the probes was constructed entirely of polysulphon (PSU); it contained an afferent and an efferent optical fibre cable and the cables for the temperature sensor. Jacks were attached for connection of the probes to the laser Doppler and temperature units (Fig. 1). Before implantation all probes were tested for water impermeability by the Swedish manufacturer Perimed in a warm water bath (40 oC) for at least three days. Also all probes were calibrated once before implantation according to the instruction of the manufacturer.

Figure 1. Probe made entirely of PSU for the measurement of intramedullary perfusion and temperature near the cortex, with integrated afferent and efferent optical fibre cables and cable for the temperature sensor, and the jacks for connection with the laser Doppler and temperature measurement units. 1: Efferent optical fibre cable, jack. 2: Cable for temperature measurement, jack. 3: Afferent optical fibre cable, jack.

The probes were 25 mm long (without cables) and 3.5 mm in diameter at the shaft. The point of the probe was 1.5 mm in diameter and 2.0 mm long (Fig. 2). The point contained the temperature sensor, an afferent and an efferent optical fibre cable. The two optical fibre cables were 250 Ìm apart. These probes were initially implanted in three of the five rabbits. The probes were later modified for the

Figure 2. Point and shaft of the probe in Fig. 1 (detail).

other two rabbits by reinforcing the rear part of the shaft with a case of medical steel (Fig. 3). The metal reinforcement was made short enough to prevent contact between the metal and the soft tissue surrounding the tibia. The temperature sensor was also made less permeable to body fluids.

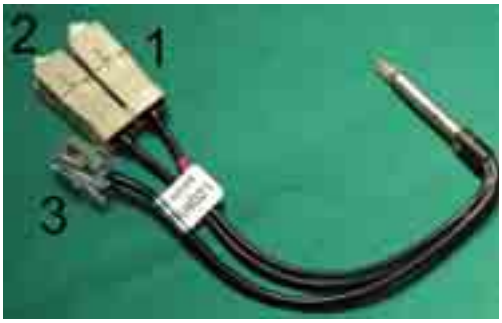

Figure 3. Probe for the measurement of intramedullary perfusion and temperature near the bone cortex, with metal reinforcement in the rear area and the jacks for connection with the laser Doppler and temperature units. 1: Afferent optical fibre cable, jack. 2: Efferent optical fibre cable, jack. 3: Cable for temperature measurement, jack.

The surgery was conducted after premedication of the rabbits with an intramuscular injection of 17 mg/kg S-ketamine (Ketanest®, Parke-Davis, Karlsruhe, Germany) and 0.25 mg/kg medetomidine (Domitor®, Pfizer, Karlsruhe, Germany). Anaesthesia was maintained with 1.5 vol% to 2.5 vol% isoflurane (Isoba®, Essex, Munich, Germany) in pure oxygen. The animals were also injected subcutaneously prior to surgery with 10 mg/kg 2.5% enrofloxacin (Baytril®, Bayer, Leverkusen, Germany) and 4 mg/kg carprofen injection fluid (Rimadyl®, Pfizer, Karlsruhe, Germany). During surgery, they were given 100 ml infusion (Pädiafusin, Baxter, Unterschleissheim, Germany) intravenously.

The right hind limb was shaved circularly from the middle of the metatarsus to the middle of the femur and the skin washed and disinfected. Surgery was conducted with the animals in dorsal recumbency. A bilateral, single-plane external fixator was implanted (Mini-Fixateur externe, Mathys, Bochum, Germany) to permit attachment of the probe. Four Kirschner-pins oscillating drilling wires (diameter 1.6 mm) were attached from medial through the right tibia about 15 mm apart from each other. These pins were then attached to each other on both sides by 3.0-mm-thick rods attached to clamp jaws. Subsequently three drillings of 1.5 mm diameter were made between the pins through the cortex into the marrow space. Clamp jaws (Perimed, Järfälla, Sweden) were later used to guide the probes onto the external fixator to make it possible to locate the drilling sites in the bone accurately. The points of the probes were then introduced into the marrow space and the distal ends were attached to the external fixator (Fig. 4 and fig. 5).

Radiographs were taken in craniocaudal projection immediately after surgery and once weekly thereafter in order to monitor the position of the probes (Fig. 5). The limb was bandaged after surgery to protect the contacts and the bandage was changed during daily monitoring and cleaning of the splint.

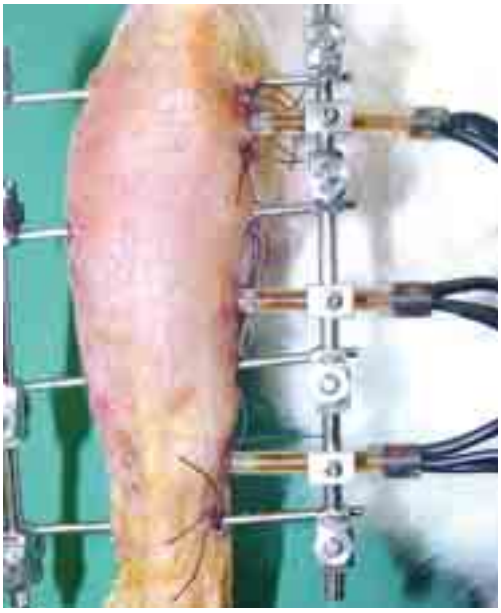

Figure 4. Tibia (dorsal view, immediately after surgery) of an experimental animal with the medially implanted PSU probes for measurement of intramedullary perfusion and temperature near the cortex. The clamp jaws for attaching the probe to the external splint are clearly visible.

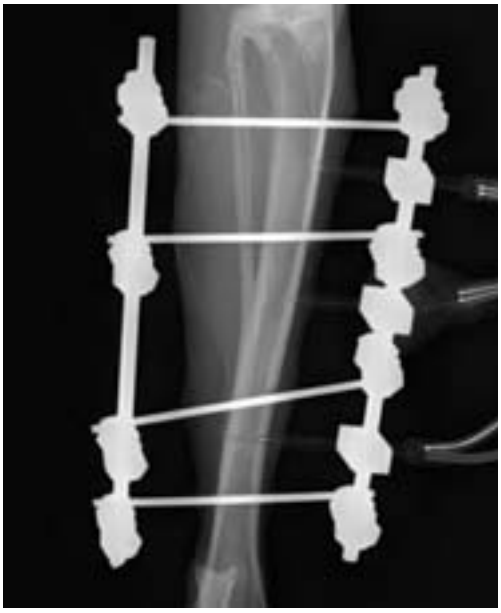

Figure 5. Tibia (radiograph, dorsoventral projection) of an experimental animal with the medially implanted PSU probes for measurement of intramedullary perfusion and temperature near the cortex.

Figure 6. Three laser Doppler PF5010 LDPM units and two PF5020 temperature units (Perimed, Sweden). Each temperature unit contains two temperature measurement channels.

Enrofloxacin and carprofen were administered in the dosages indicated above for ten more days.

Intramedullary perfusion and temperature were measured using three PDF 5010 LDPM laser Doppler units and two PF5020 temperature units, each with two channels (Perimed, Järfälla, Sweden) (Fig. 6).

Intramedullary perfusion and temperature near the cortex were to be measured at the same time every day for 49 days. The bandage was removed for the measurement and the three probes were connected at the same time by jacks with the laser Doppler and temperature units. The animals were held in semi-upright position by a technician. The measurements of intramedullary perfusion and temperature near the cortex were taken for a period of two minutes, saved on a portable computer and analysed with Perisoft 2.5 software (Perimed, Järfälla, Sweden).

The animals were euthanised painlessly under narcosis after the experiments were stopped or completed. Defective probes were returned to the manufacturer (Perimed) to determine the cause of failure.

Results

Implantation of the external fixator and the probes were tolerated well by the animals, as were the postoperative measures. No infections or other complications were observed after surgery. The conscious animals tolerated the handling involved in taking the measurements well, and it was possible to begin recording values as soon as the laser Doppler and temperature units were connected. The animals were not observed attempting to resist handling either during connection to the measuring units nor during the measurement itself.

Probes without metal reinforcement (animals 1, 2 and 3)

During the trials with the nine unreinforced PSU probes implanted in three rabbits, three probes broke on days 4, 22 and 25, so that neither measurements of intramedullary perfusion nor temperature could be made after that. Furthermore, the cables of two probes were destroyed by one rabbit (Animal 3, Tab. 1). Three probes gave no or only brief readings for intramedullary perfusion. However, one probe continued to give blood flow readings until the experiment was stopped on day 23 (Tab. 1).

Four of the nine probes gave either too high or no temperature readings (Tab. 1). Examination of the probes returned to the manufacturer indicated that both high and missing temperature readings were due to moisture having penetrated the temperature sensor.

Table 1. Duration of functionality of all the probes for measurement of intramedullary perfusion and temperature near the cortex, and time when the experiment was stopped.

		Probes without reinforcement			**Reinforced probes**	
		Animal 1	**Animal 2**	**Animal 3**	**Animal 4**	**Animal 5**
Proximal probe	**Perfusion**	22 days#	23 days	5 days■	49 days	49 days
	Temperature	7 days*	9 days*	5 days■	49 days	49 days
Center probe	**Perfusion**	25 days#	4 days#	5 days■	49 days	49 days
	Temperature	6 days	4 days	5 days■	49 days	49 days
Distal probe	**Perfusion**	-	4 days	-	49 days	49 days
	Temperature	25 days	12 days*	6 days	49 days	49 days
Day when experiment was stopped		25	23	6	49	49

Probe shaft broke

* Subsequent temperature readings of between 40 and 100 °C

■ Probe cables destroyed by animals' gnawing

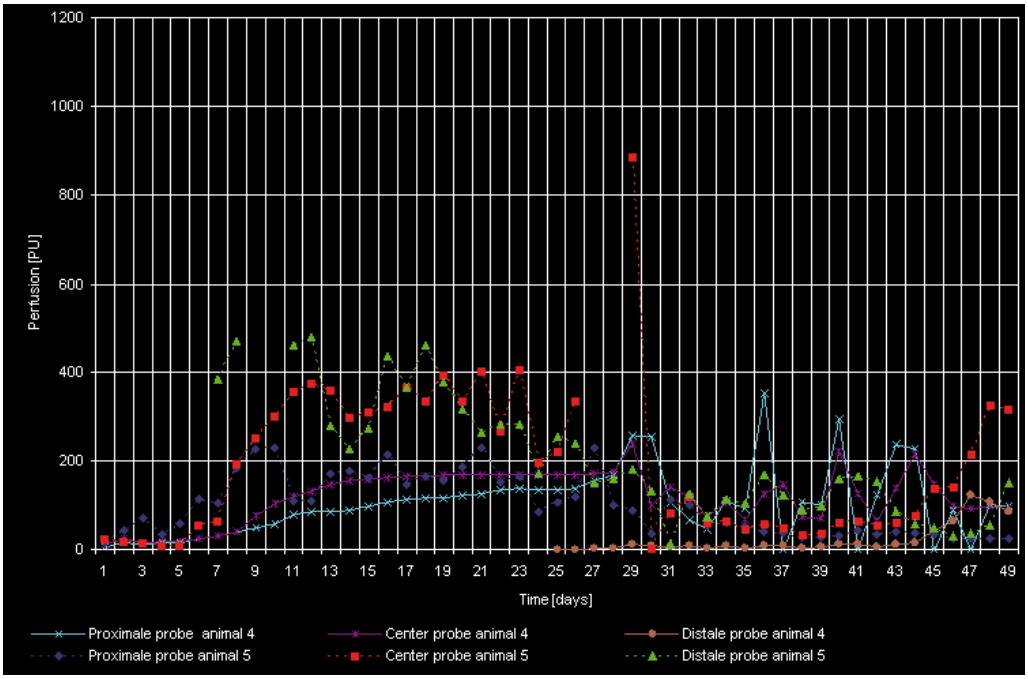

Figure 7. Record of intramedullary perfusion [PU] for 49 days in the proximal, center and distal probes of the experimental animals 4 and 5; these probes have been reinforced with metal.

PSU probes reinforced with metal (animals 4 and 5)
None of the six reinforced probes implanted in two rabbits broke. Four of these six, in both cases the proximal and center probes, gave almost continuous readings for intramedullary perfusion. However, the two distal probes did not give any readings for intramedullary perfusion until the 11th and 25th day of the experiment, respectively, but thereafter they worked flawlessly until the end of the experiment on day 49. Figure 7 shows the intramedullary perfusion during the observation period at the three probe sites of the two rabbits 4 and 5.

All six probes gave measurements for temperature near the cortex almost through the entire experimental period (Fig. 8). Only in animal 5 two interruptions of the temperature measurements were observed. The temperature could not be measured at day 10 and 11 as well as day 33 and 34 at any probe. The values of the temperature measured near the cortex varied from day two between 31.5 °C and 39.7 °C, with the distal probes giving the lowest readings.

Discussion

The purpose of this pilot study was to determine the suitability of the permanent probe implants manufactured especially for this experiment for the continuous measurement of intramedullary perfusion and temperature near the bone cortex.

The rabbit was chosen as the model animal because it was possible to compare the results of our study with a number of studies on the measurement of cortical or intramedullary perfusion with LDF or LDI in the anaesthetised rabbit (*Chan et al., 1999; Herzog et al. 2002; McDonald and Pitt Fort, 1993 and 1994; Salerud and Hellem, 1992; Shymkiw et al. 2001; Wolf et al. 2000*). The tibia was chosen because it is well suited for application of a bilateral external fixator, allowing easy implantation of the probes medially without extensive soft tissue

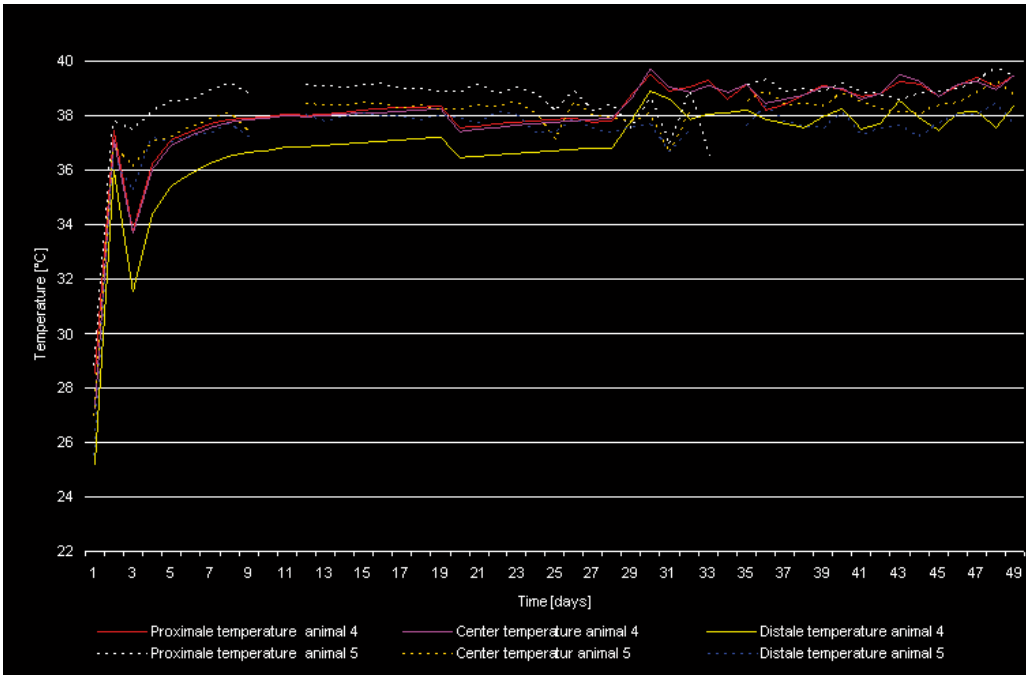

Figure 8. Record of the temperature near the cortex [°C] for 49 days in the proximal, center and distal probes of the experimental animals 4 and 5; these probes have been reinforced with metal.

damage. The external fixator held the probes firmly in place while protecting them from damage. Implanting the probes medially and covering them with a bandage also considerably reduced the danger of damage in comparison to implantation in the femur, for example.

While Jain and co-workers (*1996 and 2000*) used flexible two-way single-conductor permanent cable implants for repeated measurements in the dog, we used rigid probes with an afferent and an efferent optical fibre cable. The advantage of the latter type of probe over the two-way single-conductor cables is that it permits the light rays to penetrate the tissue in question more deeply, thus giving a greater volume of measurements (*Schemitsch et al., 1994*). It was necessary that the rabbits become accustomed to daily direct contact with humans so that the probes could be attached to the measuring instruments for the readings of intramedullary perfusion and temperature near the cortex without the animals' attempting to resist. This was particularly important, since the protective bandages were removed during the measurements, and resistance by the rabbits could have led to damage to the probes and connective cables. Unlike Jain and co-workers (*1996 and 2000*), we did not sedate the animals while they were being connected to the measuring units or during the measurement itself. In this way there was no possible influence of anaesthesia on the intramedullary perfusion or the temperature near the cortex.

Jain and co-workers (*1996*) working with dogs report that only 15% of the 55 implanted two-way single-conductor cables were still functioning after ten weeks. The optical fibre cable was found to be broken in several places in six probes which were apparently intact. However, in the vast majority of cases, the probes had been pulled out by the animals themselves, as the ends of the probes protruded through the skin. Those authors assume as possible

causes for this behaviour skin irritation at the point of exit or subcutaneous irritation (*Jain et al., 1996*). In the present study, one rabbit destroyed the cables of two probes despite the protective bandage (Tab. 1).

In a later study, Jain and co-workers (*2000*) used modified, flexible two-way, single-conductor cables for permanent implantation in dogs. After ten weeks 75% of the implanted probes were still intact. This was attributed to the modified design of the probe and the subcutaneous connections (*Jain et al. 2000*).

Similar observations were made in the present study. The probes in the first three animals were neither made of nor reinforced with metal in order to minimise the heat conductancy of the probes and prevent the probes from influencing the temperature near the cortex. PSU appeared to be a suitable material, since it has a much lower thermal conductancy (0.24 $W*km^{-1}*K^{-1}$) than for example medical steel (Material No. 1.4404; thermal conductance 16.3 $W*m^{-1}*K^{-1}$). However, three out of nine of these probes did not stand up to the demands of the animal experiment and broke at the point of attachment. Furthermore, four of those probes gave no or incorrectly high temperature readings after being penetrated by body fluids.

The modificaton of the probes by adding metal reinforcement in the rear part of the probe and by additional protection of the temperature sensors against moisture improved the function of the probes, making them suitable for the demands of the planned animal experiment. None of the implanted six probes in animal 4 and 5 broke during the 49-day observation period.

Some of the modified probes gave no readings for a few days at the beginning of the observation period respectively for few days during the observation period for this malfunction could be the presence of haematomas in the vicinity of the probes or insufficient reflected light in the optical fibre at the probe site.

None of the six modified probes gave incorrectly high temperature readings during the 49-day observation period. Since the metal reinforcement of the probes did not protrude into the soft tissue of any of the tibia bones, the metal could not have led to increased thermal conductance. The interruptions of temperature measurement in animal 5 can presumably be attributed to a malfunction of the connection between the probes and the two PF5020 temperature units. A possible explanation for the observed similar temperature distribution in both animals between the probes is the varying thickness of the covering of soft tissue along the tibia. In the proximal and center area of the tibia there is more soft tissue medially and laterally than in the distal area of the tibia. Thus, the proximal and center probes are surrounded by muscle tissue that is well supplied with blood, while the distal probe is only covered by skin that is not well perfused.

These preliminary findings indicate that these modified probes are suitable for daily measurement of intramedullary perfusion and temperature near the bone cortex in rabbits for extended periods of time. However, for statistical evaluation further investigations on intramedullary perfusion and temperature near the cortex are required in the intact rabbit tibia.

The use of these probes in a fracture model also appears possible for the continuous investigation of intramedullar perfusion and temperature near the cortex during fracture healing in the rabbit. In particular, it should be possible to study the influence of various intramedullar implants on blood flow.

An essential advantage of the model for measurement of intramedullary perfusion described here over other methods is the opportunity to obtain real-time values for intramedullary perfusion and temperature near the bone cortex without any effects of sedation or anaesthesia and without repeated surgical procedures.

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References

Chan RC, K Forrester, J McDougall, RC Bray & RF Zernicke. Dynamic measurement of bone blood perfusion with modified laser Doppler imaging. J. Orthop. Res., 1999, *17*, 578-581.

Grundnes O & O Reikerås. Blood flow and mechanical properties of healing bone. Femoral osteotomies studied in rats. Acta Orthop. Scand., 1992, *63*(5), 487-491.

Herzog L, FX Huber, PJ Meeder, G Muhr & J Buchholz.. Laser doppler flow imaging of open lower leg fractures in an animal experimental model. J. of Orthop. Surg., 2002, *10*(2), 114-119.

Jain R, N Podworny, GI Anderson & EH Schemitsch. Assessment of the relationship between standard probe and implantable fiber measurements of cortical bone blood flow: a canine study. Calcif. Tissue Int., 1996, *59*, 64-69.

Jain R., N Podworny & EH Schemitsch. In vivo assessment of second generation implantable laser Doppler flowmetry fibres for bone blood flow determination. Int. J. Surg. Investig., 2000, *2*(2) 89-98.

Kregor PJ, D Senft, D Parvin, C Campbell, S Toomey, C Parker, T Gillespy & MF Swiontkowski. Cortical bone perfusion in plated fractured sheep tibiae. J. Orthop. Res., 1995 *13*, 715-724.

Lausten GS, T Kiaer & B Dahl. Laser doppler flowmetry for estimation of bone blood flow: studies of reproducibility and correlation with microsphere technique. J. Orthop. Res., 1993, *11*, 573-580.

McDonald F & TR Pitt Ford. Blood flow changes in the tibia during external loading. J. Orthop. Res., 1993, *11*, 36-48.

McDonald F & TR Pitt Ford. The effect of blood flow on the material properties of bone. Ann. Biomed. Eng., 1994, *22*, 194-201.

Paradis GR & PJ Kelly. Blood flow and mineral deposition in canine tibial fractures. J. Bone Joint Surg. Am., 1975, *57A*(2), 220-226.

Salerud EG & S Hellem. A bone tissue integrated single fibre laser Doppler flowmeter probe. In *Steiner R, PB Weisz, R Langer* (eds.): Angiogenesis: Key Principles – Science – Technology – Medicine, 373-380, Birkhäuser-Verlag, Basel, 1992.

Schemitsch EH, MJ Kowalski & MF Swiontkowski. Evaluation of a laser Doppler flowmetry implantable fiber system for determination of threshold thickness for flow detection in bone. Calcif. Tissue Int., 1994, *55*, 216-222.

Shymkiw RC, RF Zernicke, KR Forrester & RC Bray. Evaluation of laser-Doppler perfusion imaging for measurement of blood flow in cortical bone. J. Appl. Physiol., 2001, *90*(4) 1314-1318.

Wolf K, J Hamar, E Höcherl & C Pfister. Development and reagibility of the micro-circulation in the granulation tissue after distance osteosynthesis: An experimental analysis of laser doppler flowmetry under the influence of CGRP. Appl. Cardiopulm. Pathophysiol. 2000, *9*, 333-342.