Laser doppler flow imaging of open lower leg fractures in an animal experimental model

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ABSTRACT

Purpose. Open lower leg fractures are frequently associated with severe soft tissue damage, followed by osteomyelitis. Using an animal experimental model, we investigated the effect of timing of coverage of a tibial fracture with a local muscle flap.

Methods. 80 rabbits had a tibial fracture induced in a standardised fashion, which was stabilised by screw osteosynthesis. After 3 (group A; n=40) and 7 days (group B; n=40), respectively, the tissue defect was covered by a local gastrocnemius flap. In increasing intervals from 1 to 2, 4, 8, and 16 weeks, the rabbits from each group were killed and the bone fracture was analysed histomorphologically. Cortical microcirculation was measured by 2-channel laser doppler flowmetry.

Results. Muscle flaps after 3 days improved perfusion significantly as compared with 7 days (24 Flux [standard error, 5 Flux] versus 10 Flux [3 Flux]; baseline, 1.4 Flux). Group A animals also displayed a lower rate of necrosis (0 versus 38). The incidence of osteomyelitis was higher in group B than in group A (24% versus 0%).

Conclusion. Laser doppler flowmetry was proven to be a reliable, minimally invasive means for identifying avital tissue, leading to reduction in the loss of vital bone tissue in experimental settings.

Key words: decortication; laser doppler flow; local muscle flap; open fracture

INTRODUCTION

The laser doppler flowmetry (LDF) technique measures blood flow in the very small blood vessels of the microvasculature, and is rooted in the findings of Johann Christian Doppler,1 which were first published in 1843 at the royal academy at Prague. Doppler described a phenomenon of colour shift in emitted starlight, and postulated a positive correlation between the perceived colour or frequency and the relative movement of the involved object and observer. Only with the first successful construction of a ruby laser by Maiman2 in 1960, did monochromatic light become available for further analysis of the doppler effect. Riva et al.3 was among the first to take an
integrative approach to measuring blood flow in the rabbit retina in 1972.

Laser light, however, is prone to various effects in tissue. Where part of the emitted light is reflected from the surface, another part of the light is either reflected or absorbed from deeper layers, the penetrating depth depending on the laser source applied and the tissue investigated. In 1990, Bonner and Nossal found that approximately 1% of the energy was reflected and thus scattered by erythrocytes from blood vessels in the skin. The scattering of the laser light results in a frequency broadening that, when detected by a photodetector, can be used to calculate the average speed counted in volume unit. The resulting blood flow is expressed in arbitrary perfusion units, and is calculated from the erythrocyte concentration and their average speed. Flow measurements are standardised by correcting for the Brownian motion of reference particles in a reference fluid, with Flux as the standard unit for blood flow measured.

The introduction of fibre-optic devices and improved microprobes has reduced the influence of the investigated tissue, thereby allowing dynamic investigation of the microcirculation. The first experimental and clinical experience was described by Druce as well as Kiel and Shepherd in 1990, who applied the technique to investigate skin, nasal, and intestinal mucosa. Swiontowski was the first to show the differences in perfusion of chronically inflamed and avital bone by means of LDF. Caspary et al., as well as Gramse and Saria, defined 5 Flux as biological zero for skin at 8 to 24 hours post-mortem. This value is now regarded as being due to Brownian motion.

On the basis of these findings, researchers have investigated whether LDF is a suitable means for the analysis of post-traumatic revascularisation of cortical bone. In this study, we tried to answer the same question by using tibia in a rabbit model to mimic the structure involved most frequently in open fractures in human beings.

METHODS

We used 80 inbred white New Zealand rabbits, divided into 2 groups of 40. The sample size was calculated by using the paired t test, which suggested a difference in means of 0.32 (standard deviation [SD], 0.80) and alpha-value of 0.05. All animals were kept in accordance with standard procedures; housing was provided individually in hanging wire-bottom cages at 21 ± 3°C with a 12-hour dark cycle. The rabbits were investigated monthly in accordance with the 1986 GV-

Figure 1  Local fascia-free gastrocnemius muscle flap.
at wavelengths from 780 to 810 nm, and also received the frequency-broadened reflected light from the tissue investigated. The tissue volume was represented as a spherical volume with a radius of 1.5 mm. Data were gathered and stored on a computer and subsequently analysed.

The bone was removed and analysed histomorphologically. The total vessel diameter at the corticomuscular border was evaluated by light microscopy (Fig. 3). Statistical analysis was performed with professional software (Statgraphics v.2.6; Manguistics Inc., Rockville, US). Study groups were compared by using the paired \( t \) test, with \( p < 0.05 \) as the cut-off level of significance.

RESULTS

Perfusion of the isolated cortical lid was at 3.14 Flux (SD, 1.1 Flux), which was significantly lower than that of the healthy cortical bone (21.34 Flux [4.23 Flux]) or peritoneal lining (37.87 Flux [5.55 Flux]; \( p < 0.05 \)). Microcirculation was highest in the gastrocnemius muscle, at 94.49 Flux [22.15 Flux]. Macroscopically, early covering resulted in subsequent healing under the muscle flap used for group A animals (Fig. 4). Animals in group B, on the contrary, presented with fistula and abscess formation in 9 of 10 animals (Fig. 5). Histological findings supported the LDF findings. At the level of the cortical lid or in-deep muscle layer, animals in group B showed severe inflammation and necrosis, with complete necrosis of the lid in 38 animals. After early closure and plastic reconstruction, healing and reintegration of the cortical lid took place in well-defined steps for group A animals. In contrast to group B, with an incidence of osteomyelitis of 24\%, the symptom was not observed in group A at all. Following initial trauma and fracture haematoma, necrosis prevailed in the first week after closure with
the muscle flap. Soft tissue, cortical bone, and the medullary cavity were equally affected. Granulation tissue was formed soon at the border between muscle flap and cortical bone. Capillarisation was initiated in the muscle flap and proceeded to the cortical bone, filling the structural spaces available from week 4. Between weeks 8 and 16, the fibre ratio of the connective tissue at the border between bone and muscle rose, forming a neoperiostal lining (Fig. 6).

By week 16, fibrous connective tissue had been completely replaced by laminar bone. At the same time, the irregular surface had smoothened, and by the end of week 16, the cortical lid had completely revascularised from the overlying muscle flap. It had thus been reintegrated in the tibia in a process of complete healing (Fig. 7). In 38 animals of group B, using a muscle flap after 7 days did not facilitate reintegration of the cortical lid. The reparative process was only rudimentary, whereas chronic osteitis prevailed, as did accompanying suppurative soft tissue inflammation. Greater parts of the covering muscle flap became eroded. Finally, the cortical lid was widely necrotic, and resembled all features of an avascular sequestration.

In group A samples, early covering led to a significant rise in the vessel ratio (cross-sectional, see above) to a mean increase of 23.02% (standard error [SE], 7.02%). In contrast, delayed covering increased vessel diameter by only 8.59% (3.71%) with a subsequent decline to 3.77% (1.99%) at the end of week 16 (p<0.05 at weeks 4–16) [Fig. 8].

In terms of LDF, cortical lid perfusion among animals of group A was significantly higher than that among group B. Perfusion in the cortical lid of group A animals rose from the pathological zero (3.0–5.0 Flux) to the peak at one week after covering. After 16 weeks, perfusion remained at 19.2 Flux (SE, 3.3 Flux), resembling findings at the lower range of normal cortical tissue (Fig. 9). In contrast, perfusion in the cortical lid of animals in group B increased only slightly
DISCUSSION

Our results support the idea of revascularisation of primary avascular cortical bone by covering with muscle flaps, thus facilitating ingrowth of vessels and revitalisation. In our study, revascularisation first led to a local debridement of small bone fragments, followed by osteoblastic activation. Formation of bone tissue resulted at the border between primarily avital bone and muscle tissue. Our findings support those of Smahel et al. who stated that an interval of up to 3 days is sufficient for plastic muscular covering by re-establishing medullary and periostal bone perfusion. A neoperiostal lining was built up after 8 to 16 weeks. As in the results from Wissing et al. angiogenesis was aligned centripetally to the medullary cavity.

Histological findings of cortical bone repair could be confirmed by means of LDF, where a value of below 10 Flux represented failed revascularisation and failed repair, followed by tissue destruction. Values of about 20 Flux, ranging at the lower levels of healthy cortical bone perfusion, signified successful revascularisation. Complete healing and reintegration resulted. We thus agree with Salerud and Hellem and Chan et al. in that LDF is a suitable means of monitoring cortical bone perfusion. Using 2 independent probes with online reading and analysis, matched with our histomorphological data, valid findings resulted. When delayed plastic covering could not reconstitute cortical perfusion to sufficient levels (group B), early closure increased perfusion continuously to week 16, by which time near-normal values were reached (group A). As stated by Smahel et al. a neoperiostal lining forms only after week 8 in cases of sufficient perfusion; this finding was confirmed by our investigation. Until that time, regeneration is continuing; but after week 8, failure or success may become evident. The reason for this turning point remains to be investigated. Histological findings are closely correlated to the perfusion measured, ranging from abscess formation and necrosis in group B to reconstitution in group A. Reperfusion in terms of increasing total vessel diameters also improved.

The 2-channel LDF device used in our study is already well-suited for clinical practice. In intra-operative use, the relevant border between vital and avital bone can be defined. The decision to resect instead of performing necrosection becomes objective. Loss of healthy bone is minimised and prevalence of necrotic tissue in problem wounds is avoided. Consequent application of our findings in clinical practice will strongly improve post-traumatic and perioperative management in high-grade fractures that are associated with severe soft tissue damage.
REFERENCES


