Early muscle-periosteal lesion inhibits fracture healing in rats

Stein Erik Utvåg[1], Oliver Grundnes[2] and Olav Reikerås[1,2]

We assessed the effects of muscular detachment from the periosteum on fracture healing, focusing on a muscle-periosteal lesion in the initial healing process. In 30 male Wistar rats we produced a partial osteotomy in the mid-diaphysis of the left femur which was then manually broken. All fractures were reamed and stabilized with a 1.6 mm steel pin. The animals were randomly assigned to 3 groups. In group 1, an extraperiosteal detachment between muscle and periosteum was created in the middle third of the diaphysis. In group 2, an extraperiosteal detachment was created with application of an e-PTFE sheath (Gore-Tex® expanded polytetrafluoroethylene) around the shaft between muscle and periosteum during the first 2 weeks following fracture. In group 3, the dissection was identical, while the e-PTFE sheath was installed after 2 weeks. The rats were killed after 4 weeks, and their bones were evaluated radiographically and mechanically by the three-point bending test. The fractures healed by production of external callus, and radiographs revealed various degrees of periosteal callus with a radiolucent fracture line, most evident after early muscle-periosteal isolation. The callus area was significantly smaller after early muscle isolation, compared to extraperiosteal dissection alone and later tissue isolation. Bending moment and stiffness were also less in this group than in groups 1 and 3, while fracture energy was less than in group 1. No differences in mechanical properties were detected between extraperiosteal dissection alone and late-tissue isolation. This animal study underlines the importance of early muscle-periosteal apposition for fast periosteal healing of diaphyseal fractures.

Departments of [1]Orthopedics, Institute of Clinical Medicine, University Hospital, NO-9038 Tromsoe, Norway
Tel +47 7762-6000. Fax -7164. E-mail: steinatort@rito.no; [2]Orthopedics and Surgical Research, National Hospital, NO-0570 Oslo, Norway
Submitted 98-03-04. Accepted 98-09-22

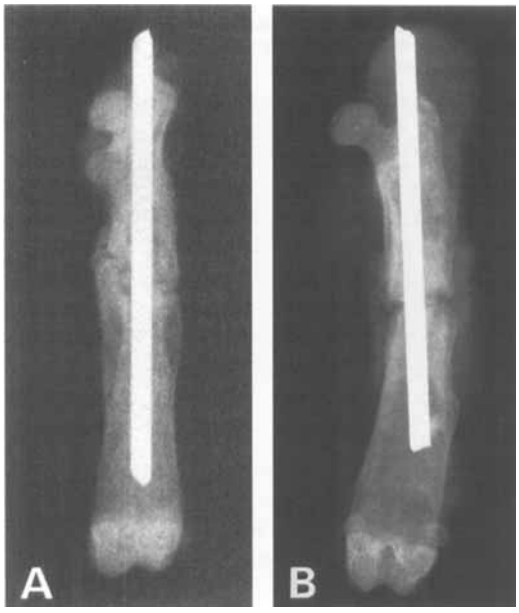
Following fracture, the periosteal cells are stimulated to proliferate and participate in the formation of callus (Einhorn 1992). However, the question of participation of cells from extraosseous tissues has not been resolved. Some authors claim that healing is accomplished almost exclusively by the activities of the periosteum and endosteum (Turek 1983), while others state that the callus forms mainly from pluripotent cells derived from several sources (Brand 1983, Rand and Bergquist 1986).

The relationship between the severity of soft tissue injury and fracture healing is well documented (Oestern and Tscherne 1984). Furthermore, fracture configuration, blood supply to the fragments and stability of fixation all have an effect on healing (Gustilo and Anderson 1976, Court-Brown and Hughes 1985). Soft tissues surrounding a diaphyseal fracture are thought to play a particular role in the healing process as a source of vascularity (Rhinelander 1974, Trueta 1974), and severe soft-tissue loss, with periosteal detachment and compromised vascularity, are major determinants of the compromised fracture healing (Gustilo et al. 1984).

In a previous study, we found that the initial repair process after femoral fracture in rats is dependent on the surrounding muscle envelope (Utvåg et al. 1998). In clinical trauma care, soft tissue coverage with a muscle flap for exposed bone is an accepted concept (Ger 1970). The present study focuses on the effects of a lesion between muscle and periosteum in the initial fracture-healing process.

Animals and methods

30 18-week-old male Wistar rats (Møllegårds Avlslaboratorium, Eiby, Denmark) weighing 347–386 g were used. The animals were housed in cages with 2 animals in each and received a standard rodent diet (Special Diet Services, U.K.; R.M. 1) with a calcium content of 0.71% and a phosphorus content of 0.5% and tap water ad libitum. The light/dark cycles were 12h/12h. The experiment conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes. The rats were randomly assigned to 3 groups in which



Healing at 4 weeks of femoral osteotomy/fracture after extraperiosteal detachment (group 1; A) and extraperiosteal detachment with application of an e-PTFE sheath between periosteum and muscle the first 2 weeks (group 2; B). Some proximal migration of the nail in B.

only installation of a membrane between muscle and periosteum was different. Following intraperitoneal anesthesia (pentobarbital 5 mg/100 g body weight, buprenophinum 0.3 mg/mL: 0.3 mL), a lateral incision of the left femur was performed. The medullary canal of the bone was entered from the greater trochanter by the use of an awl. The medullary cavity was then successively reamed to 1.5 mm by steel burrs mounted on a drill. The lateral aspect of the diaphysis was then exposed between the lateral vastus and hamstrings. A partial, transverse osteotomy at the mid-diaphyseal level was performed with a fine-toothed circular saw blade mounted on an electrical drill. The osteotomy was then manually broken. Reduction of the fracture was performed manually while a steel pin of 1.6 mm was inserted from the trochanter area through the proximal and distal fragments to the level of the condyles using an electric drill. In group 1, an extraperiosteal detachment between muscle and periosteum along the diaphyseal region from the lesser trochanteric level to the condylar area was performed in the whole circumference of the bone. In group 2, the same dissection was performed and, in addition, a 16 mm Gore-Tex® e-PTFE (expanded polytetrafluoroethylene) sheath was sutured around the shaft between the periosteal and muscular layers. In group 3, an identical dissection as in group 1 was carried out. All the rats were reoperated 2 weeks following fracture. In group 1, a redissection between muscle and periosteum was performed. In group 2, the e-PTFE membrane was removed, while redissection and installation of a membrane was performed in group 3. In this way, an identical dissection was performed in the 3 groups following fracture and at 2 weeks, while the e-PTFE sheath provided a mechanical barrier between muscle and periosteum, the first 2 weeks in group 2 and the next 2 weeks in group 3. Proper pin placement was confirmed by radiographs taken at the end of the experiment. The wounds were closed in two layers. All rats tolerated the initial operation well, resumed walking the first postoperative day and full weight-bearing after 1–2 weeks. Reoperation after 2 weeks produced no adverse effects on the function of the involved limbs.

The rats were killed in a carbon dioxide chamber after 4 weeks and the left femur was dissected free from all soft tissue. Anteroposterior and transverse diameters of the callus area were measured by a sliding caliper (accuracy of 0.01 mm). In measuring the callus, slight manual pressure was applied without indenting the callus. The quantity of the callus was expressed as total cross-sectional area, assuming an elliptical symmetry. The bones were then radiographically examined, and the intramedullary pin was removed. The bones were preserved at –70 °C between removal and mechanical testing. The mechanical characteristics of the healing fractures were tested in a cantilever bending machine. The proximal end of the femur was fixed with a clamp, the cam of a rotating wheel engaged the femoral condyles, and a fulcrum at the fracture site was the third point of force application. Refracture was performed by deflection of the distal half of the femur, as described by Engesæter et al. (1978). The testing machine was run at a constant rate of 0.08 rad/sec. The load values were documented on a chart recorder displaying the load-deformation curve. The strength was calculated as the bending moment necessary to produce refracture. The bending stiffness was determined from the slope of the linear elastic part of the curve. Fracture energy was defined as the energy absorbed during loading to refracture. In our testing situation, we experienced no slipping of clamps.

Data are presented as mean values and 95% confidence interval. For testing differences between the groups, one-way analysis of variance (Kruskall-Wallis test) was applied. When significant differences were found, the Wilcoxon rank sum test was used. The level of significance was set at $p < 0.05$.

Cross-sectionnal area of callus (mm^2), bending moment (Nm × rad × 10^{-1}), bending stiffness (Nm/rad) and fracture energy (Nm × rad × 10^{-3}) in group 1 (extraperiosteal dissection), group 2 (e-PTFE membrane first 2 weeks) and group 3 (e-PTFE membrane 2 weeks after fracture) 4 weeks after fracture. Mean and 95% confidence interval

				P-value			
	Group 1	Group 2	Group 3	1 vs 2	1 vs 3	2 vs 3	overall [a]
Callus area	80 (76–85)	28 (24–33)	64 (50–79)	0.001	0.02	0.001	0.001
Bending moment	1.31 (0.95–1.68)	0.26 (0.13–0.39)	0.98 (0.57–1.39)	0.001	0.15	0.001	0.001
Bending stiffness	5.75 (4.17–7.32)	1.22 (0.65–1.79)	5.20 (3.82–6.59)	0.001	0.62	0.001	0.001
Fracture energy	1.48 (0.83–2.12)	0.41 (0.25–0.58)	1.05 (0.40–1.71)	0.01	0.24	0.09	0.01

[a] Kruskall-Wallis test

Results

The fractures healed by production of callus. Radiographs revealed various degrees of periosteal callus around a radiolucent fracture line (soft callus) in the different groups. The calculated callus area was significantly reduced in group 2 compared with groups 1 and 3 (Table) and in group 3 compared with group 1. Bending moment and bending stiffness were significantly reduced in group 2 compared with group 1 and group 3, while fracture energy was less than in group 1. No significant differences in mechanical properties were detected between extraperiosteal dissection alone (group 1) and e-PTFE membrane superimposed on dissection after 2 weeks (group 3).

Discussion

Previous animal studies indicate that fracture healing is dependent on the surrounding muscle envelope (Richards and Schemitsch 1989, Utvåg et al. 1998). This study focuses on the effects of a lesion between the periosteum and skeletal muscles, and in which part of the initial fracture-healing process muscle coverage plays the predominant role. We found that isolation of the bone cortex/periosteum complex from the muscle envelope in the first 2 weeks after fracture reduced the capacity to form osseous tissue between the bone ends. Isolation of the muscle-periosteum interface in a later phase of healing did not significantly influence fracture healing, as compared to surgical dissection alone. This indicates that periosteal callus formation is due to a complex interaction between skeleletal muscle and periosteum, and that the integrity of the muscle-periosteum interface in the early phase following fracture is an important prerequisite for the formation of periosteal callus.

These results may have important clinical implications. In acute trauma care, diaphyseal fractures associated with high energy trauma and severe soft-tissue injury represent an important challenge, both in the treatment of soft tissue as well as in ensuing uncomplicated fracture healing (Gustilo and Anderson 1976). These fractures, frequently located in the leg region, have a high incidence of delayed union and nonunion. Investigations comparing external fixation and IM nailing indicate that the clinical outcome is largely reflected by the degree of soft-tissue injury and devascularization of fractured bone (Court-Brown et al. 1991). Soft-tissue coverage with a muscle flap for exposed bone is an accepted concept (Ger 1970), and several authors (Byrd et al. 1981, Gustilo et al. 1984) have drawn attention to the importance of early soft-tissue coverage, while the exact timing of wound closure is still debated. In determining the timing of soft-tissue coverage and wound closure, the effects on fracture healing are of clinical importance, as our study indicates a harmful effect of early isolation of the periosteum from the surrounding muscle envelope.

Previous investigators appear to exclude muscle tissue as a significant source of osteogenic cells for fracture healing (Oni 1996) and it has been assumed that the role of surrounding tissues in diaphyseal fracture-repair is to provide oxygen and nutrients (Rhinelander 1974). In our experimental set-up, the Soft Tissue Patch (1 mm thick; pore size 22 μ; Gore-Tex®, W. L. Gore & Associates Inc, Flagstaff, Arizona, USA) of expanded, fibrillated polytetrafluoroethylene (e-PTFE) blocked invasion of pluripotent cells from the muscles to reach the periosteum at the fracture site (Utvåg et al. 1998). Furthermore, invasion of blood vessels from the muscles to the periosteum was avoided. Thus, both cellular and vascular components from the muscles were prevented from reaching the fracture site.

Contrast perfusion studies employing tissue exclusion techniques have indicated that blood supply from the periosteum and soft tissue is more important than that from the marrow in the healing of long bones (Trueta 1974). However, only exclusion of the marrow circulation was studied with investigation of flow after intramedullary nailing. Further investigations isolating the periosteum and musculature indicate a marked reduction in cortical flow and ability to heal when a barrier is present between the cortex and periost (Triffitt et al. 1993). Other animal studies (Whiteside et al. 1978) indicate that neither separate periosteal stripping nor medullary reaming alter the cortical flow, but concomitant reaming and periosteal stripping eliminate blood flow in the diaphyseal cortex. Extraperiosteal dissection superimposed on the fracture induces a marked reduction in healing (Whiteside and Lesker 1978). In our model, we observed no differences in mechanical characteristics between extraperiosteal dissection alone and dissection superimposed on isolation of the muscle envelope after 2 weeks. The clinical implication is that lesions in the muscle-periosteum interface in a later phase of healing have less influence on the process of healing.

Two theories have been proposed concerning the sources of osteoprogenitor cells in the fracture-healing process. According to the first theory, osteoprogenitor cells occur only in close association with the bone surface or the bone marrow (Oni 1996), and the osteogenetic potentials of the endosteum and periosteum have been documented by many authors (Einhorn 1992, Oni et al. 1992). The alternative view is that repair tissue does not arise from specialist cells, but rather from the activity of previously uncommitted mesenchymal cells that can develop the power of osteogenesis, if given the appropriate environmental stimulus (Brand 1983, Rand and Bergquist 1986). Cells derived from tissue outside the skeleton can form bone (Friedstein 1968, Owen 1970), and implants of bone morphogenic proteins have induced bone formation in soft tissues (Urist 1965), as well as intramuscular transplantation of fracture hematoma (Mizuno et al. 1990). However, a direct contribution of extraosseous tissue in fracture healing has been difficult to demonstrate.

Long-bone fractures are associated with damage to the soft tissues surrounding the bone, even in closed fractures (Oestern and Tscherne 1984). Muscle provides an important collateral source of blood to cortical bone in both clinical (Byrd et al. 1981) and experimental studies (Richards and Schemitsch 1989). Reduction in the perfusion of muscle may result in delayed union of tibial fractures (Court-Brown and Hughes 1985). Our findings, indicating a marked reduction in mechanical properties after isolation of the muscle envelope, further show the important role played by muscle in the healing process. However, whether the reduced perfusion from muscle to periosteum is the main mechanism behind the observed reduction in healing remains open for discussion. The inability of fibroblast cells from muscle to reach the periosteal surface may be part of the mechanism. These cells, with the potential of osteoblastic transformation, may be an important factor in the healing process.

In severe open fractures associated with high energy trauma, the surgical treatment of soft tissue is important for the clinical outcome (Gustilo et al. 1984, Court-Brown et al. 1991). Most authors recommend thorough debridement of soft tissue in the acute phase and soft tissue coverage with a muscle flap for exposed bone at an early stage when risk of infection is reduced. This animal study indicates that attempts to optimize the perfusion of cortical bone, for example, by muscle coverage, is necessary at an early stage to maximize the osteogenic potential in medullary nailed fractures. This may be due to the ability of the musculature to reestablish periosteal blood flow or to contribute pluripotent mesenchymal cells differentiating into osteogenic cells in the fracture-healing process.

Brand R A. Fracture healing. In: Surgery of the musculoskeletal system (Ed. Evarts C M). Churchill Livingstone, Edinburgh 1983.

Byrd H S, Cierny G III, Tebbetts J B. The management of open tibial fractures with associated soft tissue loss: external pin fixation with early flap coverage. Plast Reconstr Surg 1981; 68: 73-9.

Court-Brown C M, Hughes S P F. Hughes external fixator in treatment of tibial fractures. J R Soc Med 1985; 78: 830-7.

Court-Brown C M, McQueen M M, Quaba A A, Christie J. Locked intramedullary nailing of open tibial fractures. J Bone Joint Surg (Br) 1991; 73: 959-64.

Einhorn T A. The biology of fracture healing. Proceedings from the 3rd Conference of Int Soc of Fracture Repair, Brussels, Belgium 1992: 1-15.

Engesæter L B, Ekeland A, Langeland N. Methods for testing the mechanical properties of the rat femur. Acta Orthop Scand 1978; 49 (6): 512-8.

Friedstein A J. Induction of bone tissue by transitional epithelium. Clin Orthop 1968; 59: 21-9.

Ger R. The management of open fracture of the tibia with skin loss. Trauma 1970; 10 (2): 112-21

Gustilo R B, Anderson J T. Prevention of infection in the treatment of 1025 open fractures of long bones: Retrospective and prospective analyses. J Bone Joint Surg (Am) 1976; 58: 453-8.

Gustilo R B, Mendoza R M, Williams D N. Problems in the management of type III (severe) open fractures. J Trauma 1984; 24: 742-6.

Mizuno K, Mineo K, Tachibana T, Sumi M, Matsubara T, Hirohata K. The osteogenetic potential of fracture haematoma. Subperiosteal and intramuscular transplantation of the haematoma. J Bone Joint Surg (Br) 1990; 72 (5): 822-9.

Oestern H J, Tscherne H. Pathophysiology and classification of soft tissue injuries associated with fractures. In: Fractures with soft tissue injuries (Eds. Tshcerne H, Gotzen L). Springer Verlag, Berlin 1984: 1-9.

Oni O O A. Callus formation during diaphyseal fracture repair. Orthop Int Edit 1996; 4 (4): 269-77.

Oni O O A, Stafford H, Gregg P J. A study of diaphyseal fracture repair using tissue isolation technique. Injury 1992; 23 (7): 467-70.

Owen M. The origin of bone cells. Int Rev Cytol 1970; 28: 212-38.

Rand J A, Bergquist T H. Fracture healing. In: Imaging of orthopedic trauma and surgery (Ed. Bergquist T H). W B Saunders, Philadelphia 1986.

Rhinelander F W. Tibeal blood supply in relation to fracture healing. Clin Orthop 1974; 105: 34-81.

Richards R R, Schemitsch E H. Effect of muscle flap coverage on the repair of devascularized tibia. An experimental investigation in dog. J Orthop Res 1989; 7: 550-8.

Triffitt P D, Cieslak C A, Gregg P J. A quantitative study of the routes of blood flow to the tibial diaphysis after an osteotomy. J Orthop Res 1993; 11 (1): 49-57.

Trueta J. Blood supply and the rate of healing of tibial fractures. Clin Orthop 1974; 105: 402-18.

Turek S L. Orthopedics: Principles and their application. J B Lippincott, Philadelphia 1983.

Urist M R. Bone: Formation by osteoinduction. Science 1965; 150: 893-9.

Utvåg S E, Grundnes O, Reikerås O. Effects of lesion between bone, periosteum and muscle on fracture healing in rats. Acta Orthop Scand 1998; 69 (2): 77-80

Whiteside L A, Lesker P A. The effects of extraperiosteal and subperiosteal dissection. I. On blood flow in muscle. II. On fracture healing. J Bone Joint Surg (Am) 1978; 60: 23-30.

Whiteside L A, Ogata K, Lesker P, Reynolds F C. The acute effects of periosteal stripping and medullary reaming on regional bone blood flow. Clin Orthop 1978; 131: 266-72.