

Periosteum and bone marrow in bone lengthening

A DEXA quantitative evaluation in rabbits

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We quantitatively studied the role of periosteum and bone marrow-endosteum during lengthening in 18 growing rabbits, comparing four surgical procedures: 1) periosteum and bone marrow preservation, 2) periosteum preservation, bone marrow destruction, 3) periosteum destruction, bone marrow preservation, 4) periosteum and bone marrow destruction. An external fixator was set on one femur, the other serving as a control. Distraction began on day 5 and stopped on day 25 (0.25 mm/12 hours). On day 30, femora were harvested with a layer of muscle. Area, bone mineral content and density were measured by dual-energy x-ray absorptiometry. Procedure 2 showed the highest increase in bone mineral content around the elongated callus (127%) compared to

procedures: 1 (81%), 3 (25 %) and 4 (–8%, i.e., resorption of bone ends). A statistically significant effect on bone formation was observed when preserving (vs. destroying): 1) periosteum, 2) bone marrow (effect observed only around the distraction gap), 3) periosteum and bone marrow in combination. Periosteum alone forms a larger callus, with more mineral content than bone marrow alone, and destruction of both results in the absence of bone formation around the distraction area. Careful preservation of periosteum is essential to bone healing. Formation of bone with a large mineral content does not require bone marrow preservation, but there is an interaction effect on healing between bone marrow and periosteum.

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Since the works of G. A. Ilizarov (1990) and Ilizarov et al. (1980, 1983), the role of bone marrow and endosteum has been intensively studied in distraction osteogenesis. The role of periosteum is less well delineated. As a result of his studies on fracture healing, Ollier (1867) pointed out the larger role played by the periosteum, compared to that of bone marrow. In distraction osteogenesis, Kojimoto et al. (1988) compared qualitatively the role of endosteum and periosteum. Removal of the periosteum, but not removal of the endosteum, markedly disturbed bone healing. We evaluated quantitatively the bone formation related to periosteum and/or bone marrow-endosteum in distraction osteogenesis.

Animals and methods

Surgical procedure

The operations were performed on 18 pure-bred immature 2.4–3.0 kg New Zealand white rabbits. One randomly chosen femur was fitted with a custom-made external fixator (Figure 1) under aseptic conditions, using xylazine, ketamine and acepromazine an-

esthesia. The contralateral femur served as a control. Prophylactic penicillin G procaine was given perioperatively and for 2 days postoperatively. A lateral surgical approach was used. Animals were allocated to four surgical groups.

Group 1: periosteum and bone marrow preservation (5 animals). The periosteum was completely elevated up to its proximal and distal insertions in the muscle and bone. External fixator pins were positioned in the bone. A subtotal corticotomy was performed with a high-speed surgical steel cutter, which preserves the bone marrow. The external fixator was set in position, and the corticotomy was completed by careful distraction. A 1-mm initial distraction gap was set.

Group 2: periosteum preservation and bone marrow destruction (5 animals). The surgical procedure used in group 1 was performed, but the bone marrow was removed with a curette up to the metaphyseal cancellous bone, the endosteum scraped off, and the marrow cavity plugged with radiopaque surgical polymethylmethacrylate.

Group 3: periosteum destruction and bone marrow preservation (5 animals). The surgical procedure used

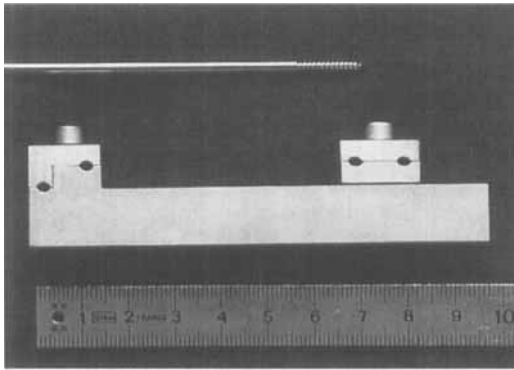


Figure 1. The external fixator used in the study.

in group 1 was performed but, after vastus lateralis elevation, the muscle fibers were progressively elevated from the periosteum. The entire diaphyseal periosteal "tube" was longitudinally sectioned and then completely removed from the bone.

Group 4: periosteum and bone marrow destruction (3 animals). The surgical techniques used in groups 2 and 3 were combined.

Each layer of soft tissue was sutured, including periosteum, when preserved, with no drainage. Animals were allowed immediate weight bearing. They were kept in individual cages, but exercised outside in an open space once a day. From day 5, the femora were lengthened 0.25 mm twice a day until day 25. On day 30, the animals were killed with an overdose of pentobarbital and CO₂ administration. After removal of the external fixator, the femora were harvested bilaterally with a 0.5–1.0 mm muscle layer, preserving the periosteum. Specimens were fixed in formalin and radiographs taken. At this stage, the percent gain measured on radiographs was 6% (the initial femoral length averaged 9.6 cm, after correction for radiographic magnification).

Preliminary study. A preliminary study was performed on the femora of 10 non-operated animals (2.4–3.0 kg New Zealand white rabbits) to determine the normal variations between the right and left sides. Both femora of non-operated rabbits were removed and fixed in the way previously described.

Dual energy x-ray absorptiometry study

Using a Hologic QDR 1000 densitometer with an ultra-high resolution V4.47 computer program for small specimens (Hologic Inc., Waltham, MA, USA), specimens were positioned in a 3-cm deep saline solution and measured anteroposteriorly three times. The specimens were removed and repositioned between each measurement. The measurement parameters were point resolution: 0.048 cm, line spacing: 0.05

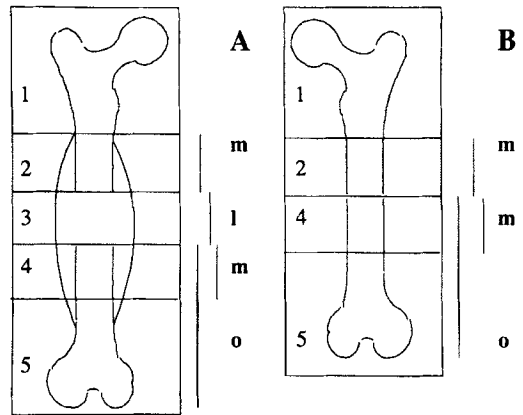


Figure 2. Regions 1–5 used for the dual-energy x-ray absorptiometry on the operated (A) and contralateral side (B). l—length of the distraction gap, m—length of the measurement around the distraction gap, o—distance from the medial condyle to the osteotomy site.

cm. The area (in cm²), bone mineral content (BMC, in g) and density (BMD, in g/cm²) were calculated.

For the preliminary study, data concerning both femora were collected. In the main study, after data collection on both femora in each animal, the distance from the medial condyle to the osteotomy site and the distraction gap length were measured. For calculation of area, BMC and BMD, the operated femur was divided into 5 regions (Figure 2): 1) proximal femoral to "m" cm from the upper edge of the osteotomy; 2) proximal edge of the osteotomy and "m" cm proximally; 3) distraction gap length (not for the contralateral femur); 4) distal edge of the osteotomy and "m" cm distally; 5) distal femur.

"m" was set at 1 cm for the shortest specimen, then adjusted for each specimen according to its total length. All external fixator pin-associated bone changes were observed in regions 1 and 5.

Statistics

The preliminary study with non-operated animals used a paired-sample hypothesis and the Student's test.

In the main study, the measurement of each region was performed for the operated and non-operated sides. The average of the 3 measurements of each specimen was used in the statistical calculations. Statistical analysis was performed on the percentages obtained:

$$\frac{\text{operated femur} - \text{nonoperated femur}}{\text{operated femur}}$$

The values ranged between -0.18 and 1.5. As negative values were observed (corresponding to resorp-

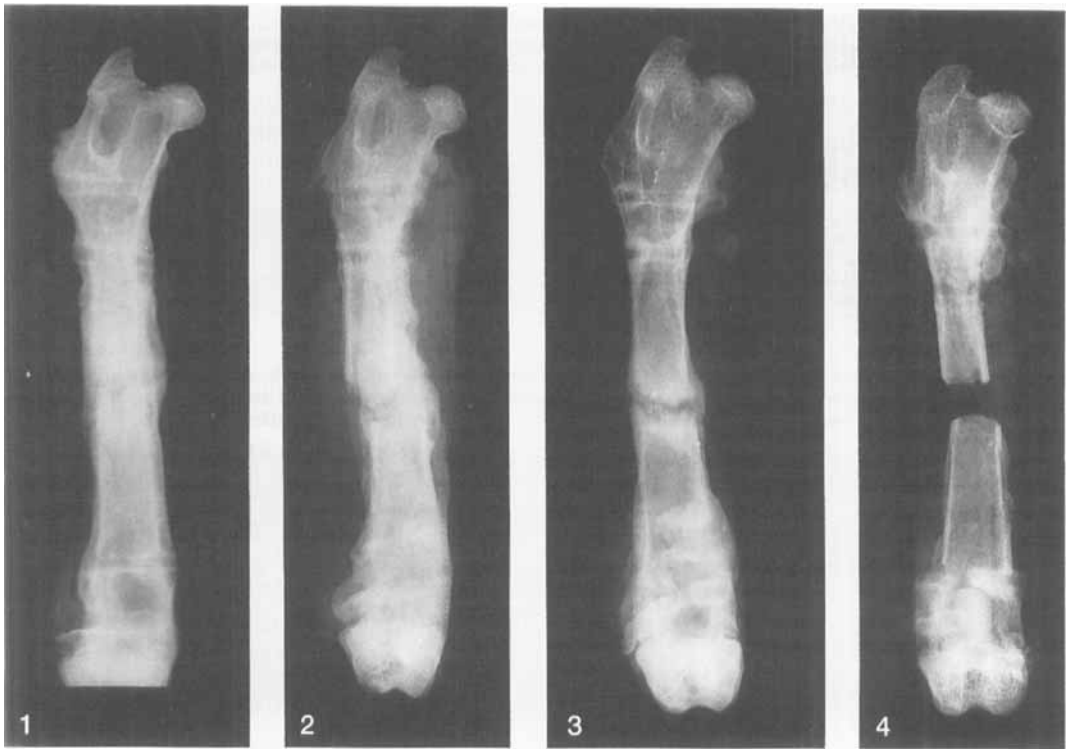


Figure 3. Anteroposterior radiographs of the operated femora (shown from left to right) from surgical groups 1 (preservation of periosteum and bone marrow), 2 (preservation of periosteum, destruction of bone marrow), 3 (preservation of bone marrow, destruction of periosteum) and 4 (destruction of periosteum and bone marrow).

tion) that were close to zero, all in group 4, these values were considered as zero. The values were then transformed (rule of three) to range between 0 and 1. An additional arcsin transformation was used to ensure normal distribution of the results obtained in percentages. The effects of periosteum, bone marrow and their interaction were studied. ANOVA and MANOVA tests were used to compare data from whole specimens (regions 1-5), and also from the regions around the distraction gap in isolation (regions 2-4, eliminating the 'pin effect', i.e., the bone remodelling around pins), with a JMP V2.0 program (SAS Institute Inc., Cary, NC, USA).

Results

Radiographic evaluation

Examination of the radiographs showed that bone formed from bone marrow was more condensed around the distraction area and that bone formation from the periosteum was spread along the diaphysis (Figure 3). When periosteum and bone marrow were destroyed, no bone deposition was seen around the distraction gap on the radiographs.

Preliminary study

Differences between right and left sides were not significant ($p > 0.05$). For BMC, actual differences averaged 1.8%, ranging from 0 to 4.1%.

Gain in area, BMC and BMD with respect to the contralateral bone

The increase (in percent) in area and BMC of the complete specimens was highest in groups 1 and 2, and lowest in group 4 (Table 1). Around the distraction gap, the maximal BMC increase for the operated femur with respect to the contralateral femur averaged 127% in the group with preservation of periosteum alone, 81% when periosteum and bone marrow-endosteum were preserved, and 25% when bone marrow-endosteum alone was preserved. In group 4, no bone formation occurred at the distraction gap (region of interest 3), and bone resorption was noted around this site (regions 2 and 4).

Statistical analysis of the transformed data

When the effect of periosteum alone was tested (periosteum preservation versus destruction, without considering the role of bone marrow), a significant difference was found for area, BMC and BMD for the

Table 1. Measurements on the whole specimens and on the three central regions of interest in the four surgical groups: mean gain (in % and 95% confidence interval) in area, bone mineral content (BMC) and bone mineral density (BMD) g/cm², for the operated femur with respect to the contralateral femur

Group	Whole specimens			Three central regions of interest		
	Area	BMC	BMD	Area	BMC	BMD
1	20 (12-24)	36 (13-50)	13 (0-22)	63 (36-80)	81 (29-136)	63 (33-96)
2	27 (19-38)	45 (33-65)	13 (9-19)	82 (57-101)	127 (113-147)	83 (65-92)
3	12 (8-15)	11 (3-25)	0 (-7-8)	29 (9-51)	25 (2-42)	47 (16-84)
4	7 (3-14)	3 (-2-8)	-4 (-6-0)	1 (-2-3)	-8 (-17-1)	-10 (-15--2)

Table 2. Effects (estimates, with p-values) of periosteum, bone marrow and interaction periosteum/bone marrow (P/BM) on the area, bone mineral content (BMC) and bone mineral density (BMD) on the whole specimens and on the three central regions of interest (around the distraction gap)

Group	Whole specimens			Three central regions of interest		
	Area	BMC	BMD	Area	BMC	BMD
Periosteum	0.57	1.16	1.37	2.13	2.08	1.54
p-value	0.0002	0.0002	0.006	<0.0001	<0.0001	0.004
Bone marrow	0.18	0.34	0.25	0.86	0.67	0.28
p-value	0.13	0.15	0.56	0.01	0.04	0.54
Interaction	-0.36	-0.55	-0.19	-1.41	-1.34	-0.91
p-value (P vs BM)	0.03	0.08	0.73	0.004	0.005	0.14

whole specimens and for the 3 central values (Table 2).

When the effect of bone marrow alone was tested (bone marrow preservation versus destruction, without considering the periosteum status), no significant difference was seen for the whole specimens. For the 3 central regions, a significant difference was found for area.

When the cross effects of periosteum and bone marrow were studied (Table 2) on the whole specimens, a significant difference was found for area (whole specimens) and for area and BMC (3 central regions).

Discussion

Dual energy x-ray absorptiometry (DEXA) is a useful tool for non-invasive evaluation of bone density and content, even in small bones (Braillon et al. 1992). In addition to quantification in a preserved and non-sectioned bone, it allows a quantitative assessment of bone healing as regards mechanical and calcium contents (Aro et al. 1989, Mazess et al. 1989, Markel et al. 1990). In vitro, the reproducibility error of DEXA is less than 1% (Rozenberg et al. 1995) and there is also a good correlation between DEXA and ash weight of femora (Braillon et al. 1992, Lu et al. 1994, Rozenberg et al. 1995).

In distraction osteogenesis, no study has previously been published evaluating quantitatively the role of bone marrow-endosteum and periosteum. Our study takes into account only the bone regeneration at 1 month postoperatively. However, we can expect similar results at later stages: if bone formation is good at 1 month in lengthening cases, clinical experience of ten shows that the bone formation will be good later on and healing will be likely. In this study, the individual effect of the periosteum is proven: it forms 5 times more bone than bone marrow. The groups with periosteum preservation (with or without bone marrow preservation) all give good bone formation (Figure 3). In groups with periosteum destruction, there was new bone formation at the bone ends which indicates that we had not completely removed the periosteum. The bone formation in relation to osteotomy was completely separated from this incidental other bone formation. Within a few months, it may be possible for the periosteum to reform completely and even form bone at the distraction site. Careful preservation of periosteum is very important at surgery.

In the study by Kojimoto et al. (1988), the bone marrow was destroyed and the endosteum was scraped off. However, such a study design may not be relevant because bone marrow and endosteum may reform within 1 week (Ollier 1867). We filled the bone marrow cavity with radiopaque surgical cement, to prevent reformation of bone marrow. This

might induce some cortical bone necrosis which would explain the resorption of bone in the group with destruction of bone marrow and periosteum. However, in the group with preservation of periosteum and destruction of bone marrow, no decrease in the bone healing response was noted. The effect of bone marrow on bone deposition is significant when measuring only the three central regions, thus eliminating the effect of remodeling around the pins. The effect of bone marrow is less important than that of periosteum. Periosteum destruction allows a callus to form from bone marrow, which may be sufficient for the healing of a distraction gap. However, Kojimoto et al. (1988) showed failure of bone lengthening when the periosteum was removed. Our results are contradictory to theirs, since we found that bone marrow alone may allow healing of the distraction regenerate.

Groups with destruction of bone marrow and periosteum failed to produce new bone around the distraction gap, and had resorption at this level. No bone formation from muscle was detected. At a longer follow-up, periosteum reformation and/or muscle might allow some bone formation. When we destroyed the bone marrow, the periosteal bone formation filled the distraction gap and could be mistaken clinically for "endosteal" bone formation. The fact that the space is filled in therefore does not directly indicate the origin—periosteal or endosteal—of that new bone.

An important finding is that the interaction between periosteum and bone marrow is significant. The effect of one modifies the effect of the other. The mechanism is not entirely understandable. It might be due to a vascular bypass, as shown by Trueta (Trueta and Cavadias 1955): if the bone marrow is destroyed, the vascular supply of the periosteum will increase, because the vascular supply of the bone marrow is suppressed. It is suggested by Trueta that the healing response of the periosteum increases, as can be seen in our study in group 2, with respect to group 1. However, it is not proven that the increase in vascular supply directly increases the bone production of the injured bone. Bone formation by periosteum or bone marrow may be under the control of various biochemical stimulators. Interactions between the substances produced by the periosteum or the bone marrow, or various feed-back phenomena, may occur and may explain a synergistic effect on bone healing. The best healing may be found when both periosteum and bone marrow are preserved, not on a quantitative level, but on a qualitative level, with its biomechanical implications.

Our findings suggest that the periosteum should be preserved in all surgical procedures, including length-

enings, and that a synergistic effect may result from the combination of periosteum and bone marrow-endosteum in bone healing.

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