# Percutaneous bone-marrow grafting of osteotomies and bony defects in rabbits

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The effects of percutaneous bone-marrow grafting done on the fifth day after osteotomies and induced defects of the shaft of the radius were studied in 41 adult rabbits. At 2 and 3 weeks, the callus volume was larger in the grafted radii than in the controls. Serial radiographs and gross and histologic findings confirmed that percutaneous bone-marrow grafting improved healing of osteotomies and defects at 4 weeks.

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Healey et al. (1990) used percutaneous bone-marrow grafting clinically for difficult delayed unions or nonunions and reported good results. Our study was undertaken with the aim to determine whether bone marrow grafted percutaneously leads to increased bone production or whether it has any effect on the early healing of osteotomies and bony defects.

## Materials and methods

A total of 41 rabbits were divided into two groups. In Group A, bilateral radial osteotomies were performed on 31 rabbits, whereas 3 rabbits were excluded—1 died of an infection, 1 died of an overdose of the anesthesia, and 1 had a fracture of both bones of the right forelimb. Out of the remaining 28 rabbits, 10 rabbits were used for weekly radiographic assessment followed by gross and histopathologic examinations at the end of 4 weeks (Table 1), and 18 rabbits were used to calculate callus volume alone at 2, 3, and 4 weeks, using 6 rabbits each time (Table 2). Group B comprised 10 rabbits in which bilateral radial bony defects were made, and all of these rabbits were used for weekly radiographic evaluation followed by gross and histopathologic examinations at the end of 4 weeks (Table 3).

### *Operative procedure*

Under anesthesia a 1.5–2.0-cm longitudinal incision was made over the center of the radius and the bone was exposed. A transverse osteotomy was made in Group A, whereas a 1-cm bony defect was made in Group B with a hand saw approximately 3 cm from the radiocarpal joint. The wound was closed in layers and dressed. No form of external splintage was used, because stability was maintained by the intact ulna. The same procedure was repeated for the other forelimb. No postoperative antibiotic was used.

Five days after the osteotomy/bony defect, each rabbit was prepared for bone-marrow grafting. Marrow was obtained from the medullary cavity of the proximal end of a randomly chosen (right or left) femur through a 19-gauge spinal needle after observing all the aseptic precautions, and 1 $cm^3$ of marrow was injected percutaneously into the osteotomy site of the right radius, while 1 cc of saline was injected similarly into the left radius for control in all the rabbits of Group A. In Group B, 2 $cm^3$ of marrow and a normal saline solution were similarly injected. The rabbits were then returned to their cages.

All the rabbits of both series (except callus volume of the animals of Group A) were radiographed on Day 1 after bone-marrow grafting, followed by weekly anteroposterior and lateral views of both forelimbs until 4 weeks, after which a detailed gross and histopathologic examination was undertaken. The slides were carefully studied histologically for the following:

1. Presence of osteoid tissue.
2. Maturity and amount of newly formed callus.
3. Presence of fibrovascular reaction.
4. Amount of fibrous tissue leading to fibrous union, fibro-osseous union, or complete osseous union.

Callus was surgically removed from the cortex of the radii of the callus-volume group animals of Group A separately from both forelimbs under a dissecting microscope, and the volume of the removed callus was

Table 1. Radiographic, gross and histopathologic observations in Group A (osteotomy)

| A | B | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $D_1$ | $D_2$ | $D_3$ | E |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1,2 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | – | 1,2 | 1,2 | 2 | 2 | 5 | 2 |
| 2 | Died (excluded) | | | | | | | | |
| 3 | 1 | 1 | 1,2 | 3 | 3 | 1 | 1 | 4 | 1 |
| | 2 | 1 | 1,2 | 1,2 | 3 | 1 | 1 | 4 | 2 |
| 4 | Died (excluded) | | | | | | | | |
| 5 | 1 | – | 1 | 1,2 | 1,2 | 2 | 2 | 5 | 2 |
| | 2 | – | – | 1 | 1,2 | 3 | 3 | 5 | 3 |
| 6 | Fracture of both bones, right forelimb (excluded) | | | | | | | | |
| 7 | 1 | 1 | 1,2 | 1,2 | 3 | 2 | 1 | 4 | 1 |
| | 2 | – | – | 1,2 | 1,2 | 2 | 2 | 5 | 3 |
| 8 | 1 | 1 | 1,2 | 3 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | 1,2 | 1,2 | 3 | 1 | 1 | 4 | 2 |
| 9 | 1 | 1 | 1,2 | 3 | 3 | 1 | 1 | 4 | 1 |
| | 2 | 1 | 1,2 | 1,2 | 3 | 2 | 2 | 4 | 1 |
| 10 | 1 | – | 1,2 | 1,2 | 3 | 2 | 2 | 4 | 2 |
| | 2 | – | – | 1,2 | 1,2 | 3 | 3 | 5 | 3 |
| 11 | 1 | 1 | 1,2 | 3 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | 1 | 1,2 | 3 | 2 | 2 | 4 | 2 |
| 12 | 1 | – | 1 | 1,2 | 3 | 2 | 2 | 4 | 2 |
| | 2 | – | – | 1 | 1,2 | 3 | 3 | 5 | 3 |
| 13 | 1 | 1 | 1,2 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | – | 1,2 | 1,2 | 3 | 3 | 5 | 3 |

Key to data, see Table 3.

Table 2. Measurement of callus volume in Group A (osteotomy)

| A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|
| 1 | 2 | 0.11 | | 0.08 | | |
| 2 | 2 | 0.12 | | 0.09 | | |
| 3 | 2 | 0.08 | 0.13 | 0.03 | 0.08 | 62 |
| 4 | 2 | 0.16 | | 0.11 | | |
| 5 | 2 | 0.16 | | 0.08 | | |
| 6 | 2 | 0.17 | | 0.10 | | |
| 7 | 3 | 0.17 | | 0.11 | | |
| 8 | 3 | 0.22 | | 0.14 | | |
| 9 | 3 | 0.15 | | 0.12 | | |
| 10 | 3 | 0.20 | 0.20 | 0.11 | 0.12 | 66 |
| 11 | 3 | 0.23 | | 0.12 | | |
| 12 | 3 | 0.24 | | 0.13 | | |
| 13 | 4 | 0.23 | | 0.20 | | |
| 14 | 4 | 0.27 | | 0.21 | | |
| 15 | 4 | 0.19 | | 0.17 | | |
| 16 | 4 | 0.26 | 0.25 | 0.15 | 0.18 | 41 |
| 17 | 4 | 0.24 | | 0.12 | | |
| 18 | 4 | 0.31 | | 0.21 | | |

A Rabbit
B Week of killing after grafting
C Callus volume measurement ($cm^3$) of right side (grafted)
D Mean value of right side
E Callus volume measurement ($cm^3$) of the left side (control)
F Mean value of left side
G Percentage increase of callus on the right side as compared with the left side

measured using a volume-displacement method in a 1-cc pipette at 2, 3, and 4 weeks after bone-marrow grafting.

Table 3. Radiographic, gross and histopathologic observations in Group B (bony defect)

| A | B | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $D_1$ | $D_2$ | $D_3$ | E |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | 1 | 1 | 1,2 | 1 | 2 | 5 | 2 |
| 2 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | 1 | 1 | 1 | 1,2 | 1 | 2 | 4 | 2 |
| 3 | 1 | – | 1 | 1 | 1,2 | 2 | 2 | 5 | 2 |
| | 2 | – | – | 1 | 1 | 2 | 3 | 5 | 3 |
| 4 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | – | 1 | 1 | 2 | 2 | 5 | 3 |
| 5 | 1 | – | 1 | 1 | 1,2 | 2 | 3 | 5 | 2 |
| | 2 | – | – | – | 1 | 3 | 3 | 5 | 3 |
| 6 | 1 | – | 1 | 1 | 1,2 | 2 | 2 | 5 | 2 |
| | 2 | – | – | 1 | 1 | 3 | 3 | 5 | 3 |
| 7 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | 1 | 1 | 1 | 3 | 1 | 1 | 4 | 2 |
| 8 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | – | 1 | 1 | 2 | 2 | 5 | 2 |
| 9 | 1 | 1 | 1 | 1,2 | 3 | 1 | 1 | 4 | 1 |
| | 2 | – | 1 | 1 | 1 | 2 | 2 | 5 | 3 |
| 10 | 1 | – | 1 | 1 | 3 | 2 | 2 | 4 | 2 |
| | 2 | – | – | 1 | 1 | 2 | 3 | 5 | 3 |

A Rabbit
B Side
1 right (grafted)
2 left (control)
*Radiographic evaluation*
$C_1$ 1 week
$C_2$ 2 weeks
$C_3$ 3 weeks
$C_4$ 4 weeks
1 presence of callus
2 partial bridging
3 complete bridging
*Gross features*
$D_1$ Adherence of soft tissues
$D_2$ Bulk of callus
$D_3$ Presence of gap
1 good
2 fair
3 poor
4 no
5 yes
E Histologic bridging
1 osseous
2 fibro-osseous
3 fibrous

## Results

The fracture line became indistinct earlier, and callus appeared early and was more abundant on the grafted side as compared with the control side. More fractures united on the grafted side at the end of 4 weeks (Figures 1 and 2; Tables 1 and 3).

Grossly, the soft tissues were found to be more adherent at the fracture or bony-defect site: the bulk of callus was more and the gap was not palpable on the grafted side when compared with the control side. Histopathologically, the osteotomy or the bony defect was found to be united by a complete or partial bony bridge on the grafted side, whereas union on the control side was either by fibrous tissue alone or fibro-osseous tissue with minimal callus formation (Figures 3 and 4; Tables 1 and 3).

*Measurement of callus volume.* The callus volume on the grafted side was about 50 percent larger compared with the control side (Table 2).

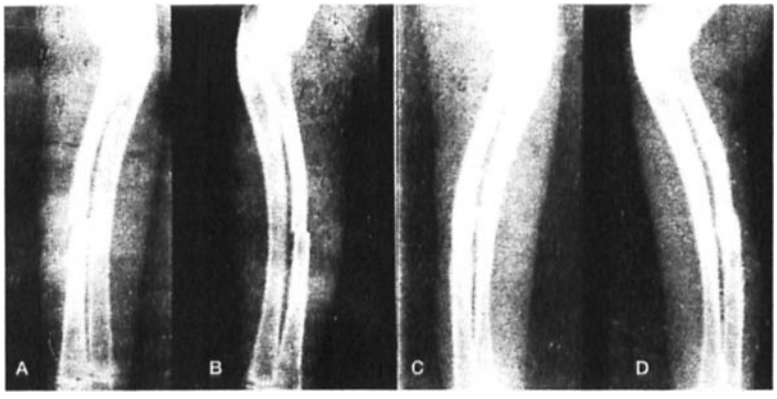


Figure 1.
A. Right forelimb, osteotomy of the grafted radius at Day 1.
B. Osteotomy of the control radius at Day 1.
C. At 4 weeks, complete union of the osteotomy of the grafted radius.
D. At 4 weeks, good callus is seen, but the fracture gap is still visible in the control radius.

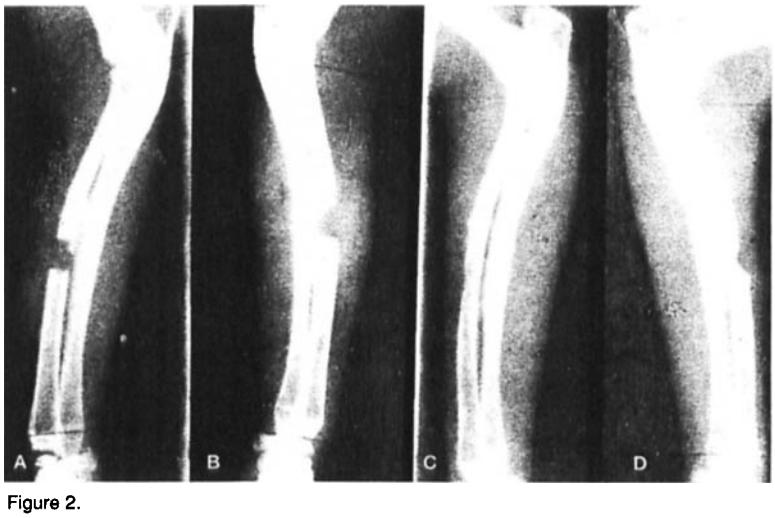


Figure 2.
A. Right forelimb, bony defect of the grafted radius at Day 1.
B. Bony defect of the control radius at Day 1.
C. At 4 weeks, complete bridging of the defect by callus in the grafted radius.
D. At 4 weeks, partial bridging of the defect in the control radius.

## Discussion

The work by Paley et al. (1986) showed that marrow produces optimal effects when used early in the fracture-healing process. In our study, bone-marrow grafting was done 5 days after performing the osteotomy or effecting the bony defect for two reasons: first, to allow the skin and subcutaneous tissue to heal so that there would not be leakage of the liquid marrow from the wound; secondly, improved grafting results may be expected by using a delayed procedure (Bassett 1972). Marrow was injected immediately after aspiration, because the number of viable cells decreases as the time span from aspiration increases (Paley et al. 1986).

Because it is known that bone marrow is osteogenic immediately after grafting (Axhausen 1956, Cummine and Nade 1977, Wittbjer et al. 1983), our study was limited to the first 5 weeks after performing an osteotomy or executing a bony defect.

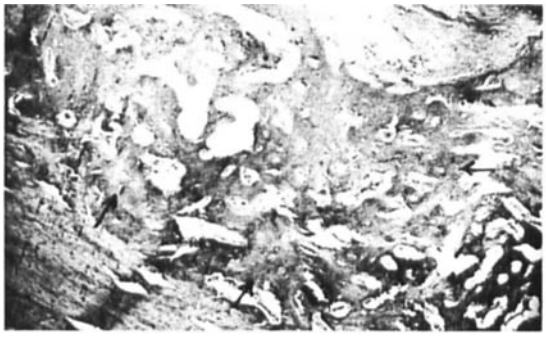

Figure 3. Photomicrograph of section of bony defect in the bone-marrow-grafted radius showing bridging of the defect by mature callus (HE, ×44).

Figure 4. Photomicrograph of section of bony defect in the control radius—showing bridging of the defect by fibrous tissue (HE, ×44).

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