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The effect of drilling parameters on bone

Part II *The influence of drilling site*

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For orthopaedic biomaterial implantation testing, specimens are often implanted into cortical bone defects. The implantation site is assumed to be one of the factors that influence the bone response to biomaterials. The aim of this study was to investigate the bone-healing process in drilled cortical defects at different sites with respect to time. Sheep metatarsus was implemented, since it is a long straight bone with four flat faces. Thus, the different drilling sites were obtained by changing the longitudinal level (proximal, middle and distal) and bone aspect (anterior, lateral and posterior). Metatarsi were obtained at 1, 2, 3 and 4 months post-operatively and with non-decalcified sections the newly formed bone area was measured using a microscope connected to an image analyser. The rate of bone formation was higher in the anterior aspect ($P < 0.05$). The new bone did not form concentrically from the hole edge towards the centre, and the principal direction of bone growth was different between the anterior and the posterior aspects ($P < 0.05$). However, there was no difference with respect to the longitudinal axis. These results indicate that the implantation site must be considered when analysing the bone response to biomaterials implanted in cortical defects.

1. Introduction

The ever-widening range of orthopaedic biomaterials requires increasing precise preclinical evaluation. In order to investigate the bone response to biomaterials, many studies have used implantation techniques in animals, often inserting cylindrical specimens into cortical defects. Cortical bone repair is influenced locally by the biomechanical conditions and blood supply [1, 2]. The size, shape and site are the factors that influence the biomechanical conditions.

When more than two specimens are implanted into one bone, they should be implanted at a certain distance in order to prevent interactions between them. However, bone is not structurally, geometrically or biomechanically homogeneous. Therefore, if the cortical bone repair process is different with regard to the defect site, the implantation site should be considered when histologically, microradiographically and mechanically analysing the bone response to biomaterials implanted in cortical defects. The aim of this study was to investigate the effect of the defect site on the bone-healing process. The bone-healing process was studied concerning the rate of bone formation and the orientation of bone growth in drilled cortical defects.

A previous study evaluated the bone-repair process in cortical defects [3]. Bone formation did not occur at a uniform rate across the entire defect. Woven bone was initially synthesized on the hole edge, and bone formation progressed towards the centre of the defect. However, whether new bone formed concentrically from the periphery of the hole to the centre has not been investigated.

To make defects at different sites in a diaphysis, sheep metatarsus was implemented. It is a long straight bone and its transverse section is nearly rectangular from top to bottom of the diaphysis [4]. Accordingly, different drilling sites were obtained by choosing the longitudinal level (proximal, middle and distal) and bone aspect [anterior (ventral), lateral and posterior (dorsal)].

2. Materials and methods

2.1. Animal experiments

Nine sheep, Préalpes du Sud, 3–6 years old and weighing about 60 kg, were used. After barbiturate injection, general anaesthesia was performed via inhaled halothane. Under sterile conditions, one metatarsus was subperiosteally exposed. A hole was drilled perpendicular to the cortical surface using a 4.0 mm outside diameter drill under irrigation with physiological saline. Three holes were created in a metatarsus, one in each segment of the diaphysis; proximal, middle and distal. Each hole was randomly distributed on three aspects; anterior, lateral and posterior (Fig. 1). Specific reapposition of the periosteum was not performed after drilling. The underlying soft tissue and

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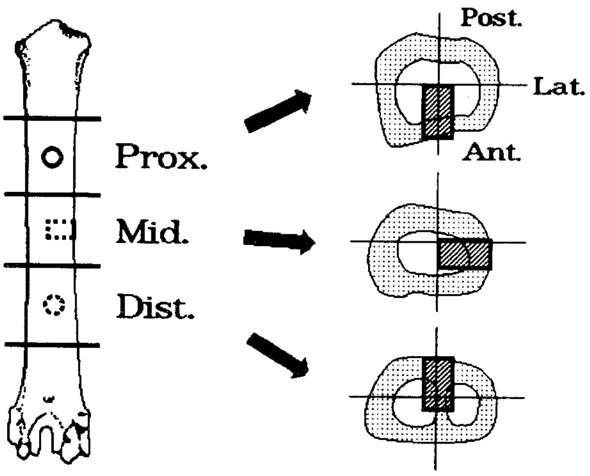


Figure 1 Example of the drilling sites in the diaphysis of sheep metatarsi. In this case one hole was drilled in the proximal segment of the anterior aspect, one in the middle segment of the lateral aspect and one in the distal segment of the posterior aspect. The combinations of the segment and the aspect were randomly distributed.

the skin were closed layer by layer. Immediate weight bearing was allowed. After several days post-operatively the sheep could walk normally without limping. All sheep were given prophylactic doses of antibiotics (600 000 units penicillin) for 5 days following surgery. The other side was operated later to be used for a different study interval.

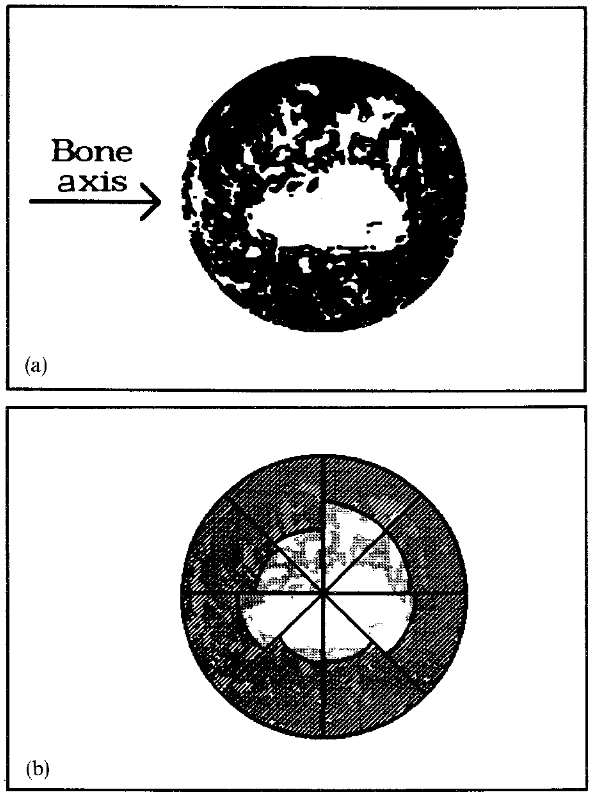
The sheep were killed at 1, 2, 3 or 4 months post-operatively. In total six 1 month, six 2 month, three 3 month and three 4 month metatarsi were available. After fixation in buffered formalin, bones were dehydrated and embedded in methylmethacrylate. The metatarsi were each cut into three pieces, each containing one hole. Using a diamond saw, the samples were sectioned perpendicular to the drilling axis, parallel to the cortical surface. One section was obtained from the middle level of the cortical thickness. Sections were ground to 150 μm and stained with Paragon.

2.2. Histomorphometry

With an optical microscope connected to an image analyser (CUE-2, Olympus Corporation, Tokyo, Japan), the area of newly formed bone in the drilled hole was measured (Fig. 2a). Furthermore, the hole was divided into eight equal fan-shaped sections. The area of newly formed bone in each section was computed in order to investigate the predominant orientation of bone growth (Fig. 2b). When the amount of newly formed bone in each fan-shaped section was replaced by the same amount of area of peripheral zone in the hole, it revealed a quasi-elliptical shape, indicating a non-homogeneous pattern of bone growth. In order to investigate the predominant orientation of bone growth mathematically, a least-squares method was applied to fit the data into an elliptical shape (Fig. 2c). The angle of the short axis of the ellipse indicates the direction of main bone growth. The bone distribution of the left limbs was symmetrically reversed in order to fit the right limb hole distribution, and all data were calculated as if all bones were right limbs. Students *t*-test was implemented throughout the statistical analysis.

3. Results

At 1 month post-operatively new bone had already begun to form and apposed on the hole edge (Fig. 3). Then woven bone continued to form towards the centre and about 58% of the drilled hole was occupied



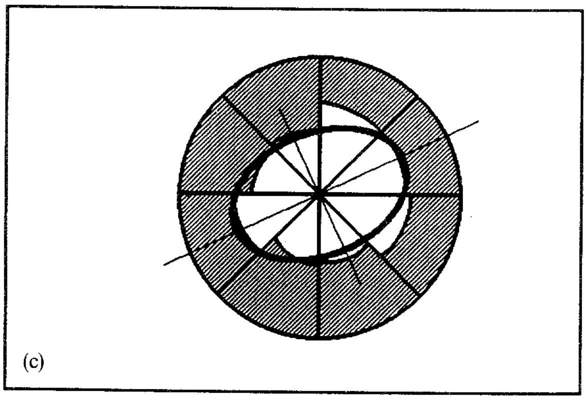


Figure 2 Example of the measurement of the newly formed bone area and the determination of the predominant bone growth orientation in the hole. The light microscopic section shown in Fig. 4 was used for this example. This hole was located at distal segment, anterior aspect in the left limb. (a) Image after being thresholded by the image analyser. The black area corresponds to the newly formed bone. (b) After division into eight equal fan-shaped sections, the area of the newly formed bone in each section was computed and is represented as the shadowed zone at the periphery of the hole. (c) The ellipse was calculated by a least-squares method. The angle of the short axis indicates the direction of main bone growth. Since this hole is in the left limb, the result was calculated to 69° to fit the right limb hole distribution.

at 2 months (Fig. 4). At 3 months the hole was nearly completely filled with newly formed bone and the peripheral part began to remodel into lamellar bone (Fig. 5). At 4 months most parts underwent internal remodelling and the boundary of the hole was hardly detectable (Fig. 6).

The area of newly formed bone was calculated as the percentage of the drilled hole area. The percentage area with regard to its position is represented in Table I. There was no difference among these positions at each experimental period. When the percentage areas were plotted, the bone formation curves showed a quasi-sigmoidal form (Fig. 7a). A best-fit computation using a least-squares method was performed with the asymptotic equation

$$y = 100\{1 - \exp[-k(t - t_0)]\} \quad (1)$$

100 indicates the asymptotic values (100% of ingrowth in the long term), and k provides the rate of bone growth with time. t_0 is the time when the bone formation process starts. Equation 1 was transformed as

$$\log(100/100 - y) = k(t - t_0) \quad (2)$$

In order to compare k, data were recalculated as $\log[100/(100 - y)]$ and the regression line was computed (Fig. 7b). Concerning the longitudinal axis, there was no statistical difference among proximal, middle and distal. However, the gradient between the anterior and posterior aspects was significantly different ($P < 0.05$), indicating that the rate of bone formation was higher in the anterior aspect (Table II). The amount of bone formation was too low at 1 month and the bone growth pattern did not show a quasi-

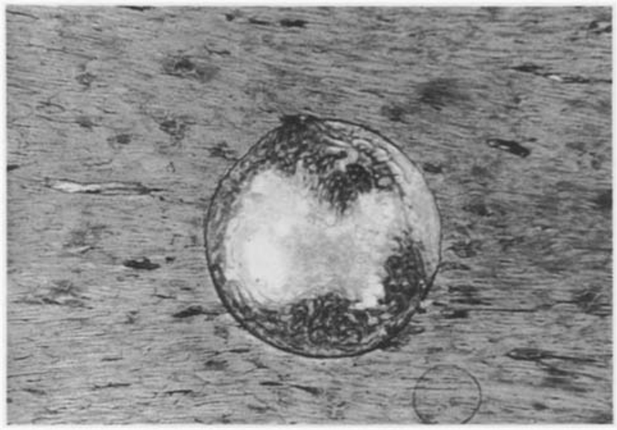


Figure 3 Photomicrograph of a 1 month specimen (Paragon stain, × 4.4). New bone began to form from the periphery towards the centre.

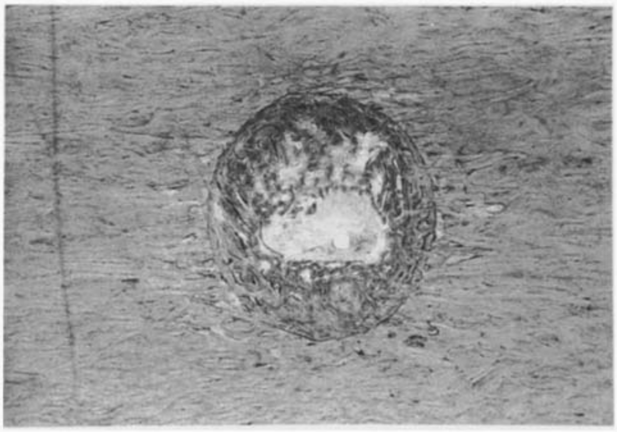


Figure 4 Photomicrograph of 2 month specimen (Paragon stain, × 4.4). Woven bone continued to form to fill the drilled hole.

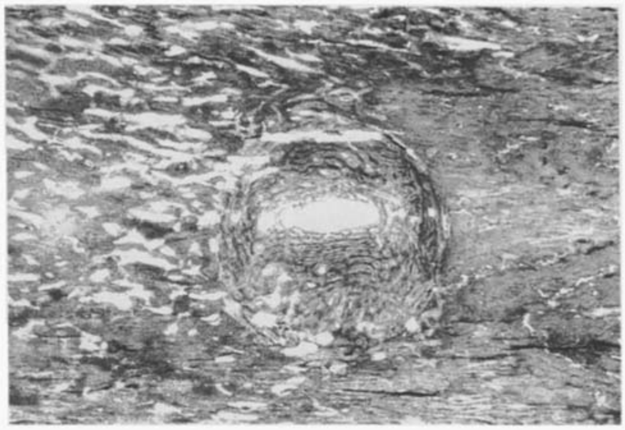


Figure 5 Photomicrograph of 3 month specimen (Paragon stain, × 4.4). The hole was almost filled with newly formed bone. The peripheral part was resorbed and began to remodel into lamellar bone.

Figure 6 Photomicrograph of 4 month specimen (Paragon stain, × 4.4). The remodelling was apparently observed all over the drilled hole.

TABLE I Comparison of new bone formation in drilled holes

Site	Percentage of newly formed bone at			
	1 month	2 months	3 months	4 months
Segment				
Proximal	6.9 ± 8.4	53.4 ± 16.2	79.3 ± 9.2	88.6 ± 8.8
Middle	10.0 ± 11.7	60.9 ± 20.1	83.4 ± 4.8	80.9 ± 1.5
Distal	3.9 ± 4.3	61.3 ± 19.6	63.7 ± 18.9	79.4 ± 6.2
Aspect				
Anterior	6.4 ± 9.6	58.5 ± 14.6	83.1 ± 2.5	85.4 ± 4.9
Lateral	4.8 ± 4.9	64.0 ± 21.3	75.4 ± 14.2	84.0 ± 11.3
Posterior	9.7 ± 10.9	53.1 ± 19.0	67.9 ± 20.3	79.4 ± 3.1

The number of specimens was six for each 1 and 2 month drilling site. The number was three for every 3 and 4 month drilling site. The values are means ± standard deviations.

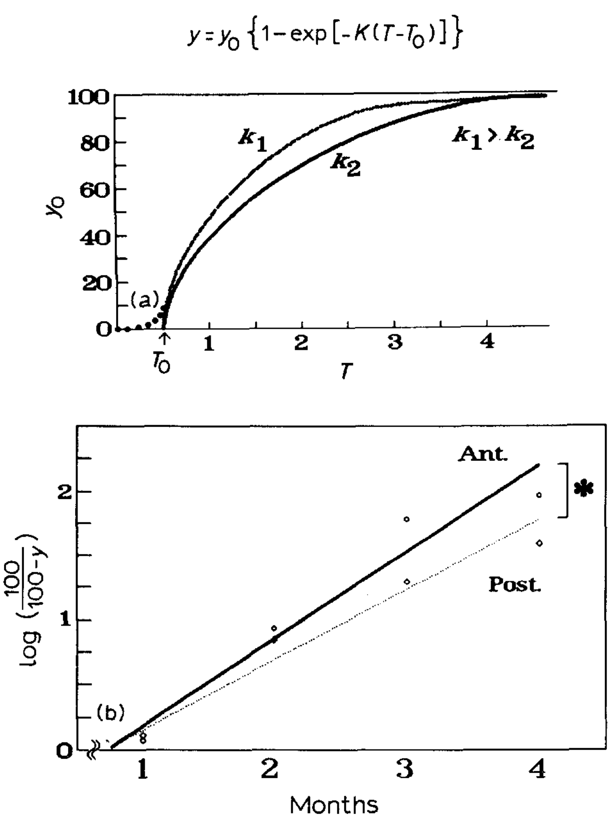


Figure 7 Method of evaluation of the bone growth rate. (a) The asymptotic exponential equation showed a sigmoid curve. When $y_0 = 100$ that indicates the 100% of bone formation at long time, the curve begins from 0 and approaches 100. k corresponds to the gradient of the curve and provides the rate of bone growth. t_0 indicates the time when the bone formation process started. The dotted curve at the left-hand end of the curve indicates the actual bone formation. (b) After recalculation using this equation, the regression lines were calculated and the gradient of each line, k, was compared. $*P < 0.05$.

TABLE II Comparison of the gradients (k) that indicate the rate of bone formation

Aspect	Gradient, k (month^{-1})	t_0 (months)
Anterior	0.67 ± 0.07	0.75
Lateral	0.68 ± 0.14	0.69
Posterior	0.51 ± 0.09*	0.59

* $P < 0.05$ versus anterior. The values are means ± standard deviations.

elliptical shape. At 3 and 4 months the healing stage of new bone formation had already finished and the calculated elliptical shape did not correspond to the preferential bone growth orientation. Therefore, the principal directions (maximum and minimum) of bone growth were computed using the 2 month sections only.

With regard to the longitudinal axis, there was no statistical difference in the predominant orientation of bone growth. However, the angle of the bone growth direction was significantly different between the anterior and the posterior aspects ($P < 0.05$), and for the lateral one it was in between these two (Fig. 8).

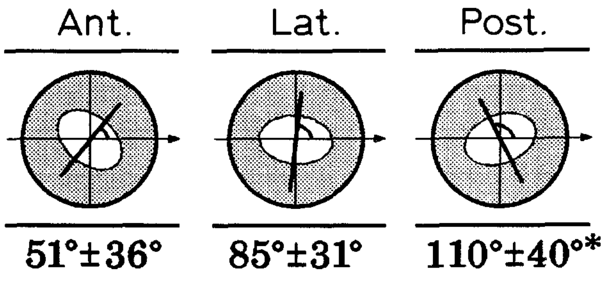


Figure 8 Comparison of the direction of predominant bone growth (2 months post-operatively). The views are looking from the periosteal surface in a radial direction. $*P < 0.05$ versus anterior.

4. Discussion

The bone geometry, biomechanical conditions and blood supply are different depending on the site of the bone. Looking at transverse sections of sheep metatarsus in detail, the cortical thickness of the proximal level is thin and that of the middle level is relatively thick. At the distal level a sagittal septum exists, which divides the marrow cavity into two. The cortical thicknesses of the four metatarsal aspects are almost the same at each longitudinal level.

Three tendons pass by the metatarsus, two dorsally and one ventrally. Since no tendon and no muscle attaches, the blood supply is not interfered with by special vessels where muscles and tendons are attached [5]. Sheep metatarsus is assumed to be nourished mainly by its nutrient vessels.

Accordingly, concerning the geometry and the blood supply, the differences between the bone aspects are assumed to be smaller than those along the longitudinal axis. In the present study the bone-healing process in cortical defects did not differ among three segments (proximal, middle and distal). However, with regard to the aspects, the rate of new bone formation and its orientation were different. Thus, the differences observed between the aspects may be influenced by other factors.

The biomechanical strains of sheep metatarsus have been investigated by several authors. Bergmann [6] studied the sheep gait and showed that the sheep metatarsus is posteriorly inclined during most of the weight-bearing phase. Claes [7] measured the *in vivo* dynamic strains of sheep metatarsus during walking. Strain gauges were set on the anterior and medial surfaces. On the anterior surface the compression stresses were recorded during the weight-bearing phase and low tensile stresses were recorded just at the end of the weight-bearing phase. On the medial surface the recorded stresses were much lower. The authors have considered that the sheep metatarsus is predominantly subjected to antero-posterior bending stress.

The mechanical properties of sheep metatarsus at various regions have been studied [8]. The moduli of elasticity among the various regions of the cross-sections was more different than that along the longitudinal axis. The highest values were measured at the ventro-medial aspect, and the lowest values were measured at the dorso-lateral aspect. Accordingly, it is assumed that the biomechanical conditions are greatly different between the aspects.

In the present study the rate of new bone formation was lower on the posterior surface, which is subjected to tensile stress. The principal direction of bone growth was different between the anterior and the posterior aspects, and the biomechanical stresses were in the opposite direction between them. It is assumed that the bone-healing process in cortical defects may be influenced by the biomechanical conditions.

Acknowledgements

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