Anatomy of the Intracortical Canal System

Scanning Electron Microscopy Study in Rabbit Femur

Ugo E. Pazzaglia MD, Terenzio Congiu MD,
Mario Raspanti MD, Federico Ranchetti MD,
Daniela Quacci MD

Received: 25 July 2008 / Accepted: 9 March 2009 / Published online: 28 March 2009
© The Association of Bone and Joint Surgeons 2009

Abstract The current model of compact bone is that of a system of longitudinal (Haversian) canals connected by transverse (Volkmann's) canals. Models based on histology or microcomputed tomography lack the morphologic detail and sense of temporal development provided by direct observation. Using direct scanning electron microscopy observation, we studied the bone surface and structure of the intracortical canal system in paired fractured surfaces in rabbit femurs, examining density of canal openings on periosteal and endosteal surfaces, internal network nodes and canal sizes, and collagen lining of the inner canal system. The blood supply of the diaphyseal compact bone entered the cortex through the canal openings on the endosteal and periosteal surfaces, with different morphologic features in the midshaft and distal shaft; their density was higher on endosteal than on periosteal surfaces in the midshaft but with no major differences among subregions. The circumference measurements along Haversian canals documented a steady reduction behind the head of the

cutting cone but rather random variations as the distance from the head increased. These observations suggested discontinuous development and variable lamellar apposition rate of osteons in different segments of their trajectory. The frequent branching and types of network nodes suggested substantial osteonal plasticity and supported the model of a network organization. The collagen fibers of the canal wall were organized in intertwined, longitudinally oriented bundles with 0.1- to 0.5- μ m holes connecting the canal lumen with the osteocyte canalicular system.

Introduction

The currently accepted model of compact bone organization is that of a system of longitudinal canals (Haversian canals) connected by transverse connections (Volkmann's canals); the blood flow supporting the osteocytes runs in this system of canals [32]. During bone development, the system of Haversian canals and the related vessel network are created by cutting cones with osteoclasts at the head and the vascular loops that follow at a short distance [26]. However, the Haversian system represents only part of the more complex lamellar architecture of the long bone diaphysis [10, 28, 29], and other lamellar systems are associated with canals and vessels connecting with the Haversian system, in particular those derived from periosteal and endosteal apposition.

The application of different methods of study based on histology [5, 9, 18, 30, 31] or micro-CT [6, 7] has led to several three-dimensional models of the canal network. These reconstructions can be useful to give a general view of the architecture of the system but are limited by the loss of morphologic details and the sense of temporal development. Specifically, longitudinal advancement of

One or more of the authors (UEP, DQ) have received funding from the University of Brescia and University of Insubria. Each author certifies that his or her institution has approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research. This work was performed at Spedali Civili di Brescia and at Dipartimento di Morfologia Umana dell'Università dell'Insubria.

U. E. Pazzaglia (✉), F. Ranchetti
2a Divisione di Ortopedia e Traumatologia, Spedali Civili di
Brescia, Clinica Ortopedica dell'Università di Brescia, Brescia,
Italy
e-mail: ortopedia2@spedalicivili.brescia.it

T. Congiu, M. Raspanti, D. Quacci
Dipartimento di Morfologia Umana, Università dell'Insubria,
Varese, Italy

Haversian canals typically is obtained by serial cross-sectioning or by mineral density analysis (in micro-CT), resulting in inferior reconstructions compared with direct observation.

Our purpose was to characterize the organization and structure of the intracortical system of canals using direct scanning electron microscopy (SEM) observation (1) to determine the density of canal openings on the periosteal and endosteal surfaces, (2) to count and classify the internal network nodes and to stereometrically assess canal size, and (3) to describe the collagen fibers bundles lining the inner canals system. These data provide the basic structural knowledge to understand changes with aging or pathology of cortical bone.

Materials and Methods

We studied the origin of the vascular canals on the periosteal/endosteal surface of the cortex with direct SEM observation and the distribution and structure of the canals inside diaphyseal bone by producing paired fractured surfaces of the cortex. This method not only provided a sufficiently broad and direct representation of the canal network with its ramifications but also allowed counting of branches, classification of network nodes, and three-dimensional morphometry to assess canal sizes with a degree of accuracy not obtainable by other methods [16, 17].

We obtained the femora of six male New Zealand White rabbits from Stefano Morini SAS, S. Polo d'Enza, Italy (mean weight, 3.0 kg; age, approximately 8 months). A rabbit of this age can be considered a mature adult because there are no additional weight increases; however, there is no growth plate cartilage closure in rodents. The animals were housed in individual cages with food and water *ad libitum* and kept in an animal house at a constant temperature of 22°C with a 12-hour light-dark cycle. Care and use of experimental animals was consistent with procedures and regulations of the Italian Health Ministry.

The rabbits were euthanized with an overdose of ketamine chloridrate (Imalgene[®]; Mevial Italia SpA, Assago, Italy) and xylazine (Rompum[®]; Bayer AG, Leverkusen, Germany). Both femurs were disarticulated at the level of the hip and knee. The left femurs were dissected, but a layer of approximately 3 mm of soft tissue was left around the whole surface of the bone. These specimens then were fixed in neutral formalin (10%) for 1 week and transferred for 4 weeks to a bath of 40% hydrogen peroxide solution in water at room temperature to remove all soft tissue without manipulation with sharp blades.

Each bone then was cut at the midlength of the shaft in a plane perpendicular to the long axis of the diaphysis with a low-speed circular saw (IsoMet[®]; Buehler Ltd, Lake

Placid, NY) and the proximal part was discarded because the lamellar organization and the system of canals cannot be compared with other levels of the diaphysis. In the distal half of the femur, we made another cut, parallel to the first and at the level of the metaphysis 2 to 3 mm below the growth plate cartilage. The prepared specimens with the shape of a cylinder were split with a cut in the median frontal plane into a dorsal and ventral hemicortex. They were cleaned in an ultrasound bath for 10 minutes, dehydrated in ascending grades of ethanol for 10 minutes at each passage and 20 minutes twice in absolute ethanol, subjected to critical-point drying in CO₂ according to standard procedures for SEM processing, and left to dry in air at room temperature for 3 days.

The 12 hemicortices were mounted on a special holder, which allowed turning the specimen 180° in such a way that the whole endosteal and periosteal surface of the same hemicortex could be observed under the SEM. Each holder then was fixed on stubs with conducting tape, coated with a thin layer of gold in a Emitech K550 vacuum sputter (Emitech United, Ashford, Kent, UK), and studied with a FEG XL 30 SEM (Philips, Eindhoven, The Netherlands). The six left femurs were used to study the surface and the six right femurs were used to produce the paired fractured surfaces.

Because the endosteal surface is concave and the periosteal convex, scanning the surfaces of the six left femurs was performed at low enlargement ($\times 35$) along three longitudinal strips (Fig. 1). Along each strip, the fields examined were uniformly distributed from the proximal (midshaft) to the distal end of the specimen (distal shaft). During scanning of each strip, the specimen was tilted to direct the electron beam as perpendicular as possible to the examined surface. The operating conditions of the SEM were 15 Kv (spot 3) at a working distance of 15 mm. Each scanned field measured 3.45 mm $\times$ 2.58 mm. The area of each field was measured and the number of canal openings on the surface was counted; then the density of the canals (number/mm²) of each field was calculated.

We assumed a rounded or elliptic black spot present on the surface corresponded to one canal as this is where electrons are not picked up by the detector of the microscope; all possible artifacts give an excess of the signal but never a black spot. We encountered no problems counting the canals that opened directly on a smooth endosteal or periosteal surface (Type A). However, when the surface was rough and irregular canals opened inside crevices or niches whose sharp and undermined edges could mask the underlying holes (Type B), we counted only the evident black spots inside the crevice or niche.

The examined fields were further distinguished in three groups or subregions according to the level of the diaphysis based on previous studies on the vascular supply of the

Fig. 1A–B The diagrams illustrate the position of the scanned fields on the (A) periosteal and (B) endosteal surfaces of each hemicortex and the topography of midshaft (M-S), middle (M), and distal shaft (D-S) subregions.

cortex [22, 23] (Fig. 1): (1) the two most proximal rows corresponded to the midshaft, (2) the two most distal to the distal shaft, and (3) those between these two levels were designated as in the middle zone. We compared the density distribution of canals between the three levels of either the endosteal or the periosteal surface and at each level between the corresponding endosteal and periosteal levels.

For each bone (the six left femurs), we determined the mean density of six fields for the midshaft and distal shaft levels and 15 fields for the middle zone; the reported density for each level and surface (endosteal/periosteal) then was calculated for the mean of the dorsal and ventral hemicortices.

To observe the fractured surfaces, the six right femurs were dissected completely from their soft tissues and fixed in neutral formalin solution (10%) for 1 week. Each bone was cut at the middiaphysis of the shaft perpendicular to the long axis. We made additional cuts parallel to the first (Fig. 2). The 12 diaphyseal cylinders designated A and C (both segments corresponding to the midshaft) were split longitudinally in four segments (Fig. 2A–B) and each of the 48 transversely cut surfaces notched (Fig. 2C) with a chisel and then fractured open with two flat-nosed pliers (Fig. 2D) so that the convex surfaces came in contact. The two convex surfaces of the two halves of each segment then were glued together with cyanoacrylate adhesive (Attac; Henkel SpA, Milano, Italy) in such a way that the two fractured surfaces appeared side by side and at the same level (Fig. 2E). The assembled specimens were transferred for 5 days to a bath of hydrogen peroxide to remove all soft tissue without mechanical manipulation. They also were cleaned in an ultrasound bath, dehydrated in ascending grades of ethanol, subjected to critical-point drying in CO₂, coated once with gold palladium as described previously, and dried in air. An additional six cylinders from the distal shaft of the femur designated as B were obtained with two transverse cuts at 4 and 10 mm from the distal growth plate cartilage [23]. These 24

Fig. 2A–E The diagrams illustrate the transverse cut levels along the femoral diaphysis and how the paired fractured surfaces were prepared: (A) a cylindrical segment of midshaft or distal shaft; (B) longitudinal sawing in four pieces; (C) notching to facilitate and guide fracture; (D) a fracture; and (E) pairing of fractured surfaces.

specimens were assembled and processed in the same mode, secured on the stubs with conducting tape, coated with a gold layer in a vacuum, and observed with SEM in the direct mode.

We examined 18 mirror-like fractured surfaces of the distal shaft and 35 of the midshaft (6 and 13, respectively, lost in processing). The latter included in the fracture plane one or more longitudinal Haversian canals whose length was sufficiently ample to assess the density of branches and the type of nodes. In total, we evaluated 54 intracortical canals. This type of evaluation was not possible with the short, branched tunnels of the periosteal-derived canal system of the distal shaft.

We recorded the density of branches; because the canals formed a network with a prevailing longitudinal pattern (the meshes of the network are longer than large), it was not possible to identify unequivocally the linear, anatomic unit corresponding to the Haversian canal of the traditional histologic description. We selected a sequence of consecutive internodal segments as straight as possible, uniform in size, and extending between the points of entry and exit from the fractured plane, as the reference length for counting the nodes. The density was expressed as a function of length (number/mm) (Fig. 3). We designated as nodes those points where branches merge or from which branches originate. They were assessed by classifying canal network nodes according to the number of arms in three classes, and the relative frequency of each class in the midshaft and distal shaft specimens of the cortex was calculated. When an arm converged in the node with a direction perpendicular to the fracture plane, it appeared as a hole (Fig. 4).

Because the edges of asymmetric hemicanals are hidden from the scan of a segment of the hemicircumference, to

Fig. 3 SEM of a fractured surface of the cortex shows the network of Haversian canals lying in the fracture plane. Each canal is selected arbitrarily as an anatomic unit with its points of entry and exit from the fracture plane; canal length was measured between these two points (white lines). The asterisks correspond to the cortical surface.

stereometrically measure the canal circumference, the fracture plane needed to divide the canal into two hemitunnels of approximately the same width and depth [14]; 28 Haversian canals were suitable for this analysis. They were distinguished in two groups: those coming to an end with a cutting head and whose direction of advancement was certain (Group A, $n = 7$) and those without a cutting head and where a direction was arbitrarily assigned (Group B, $n = 21$) (Fig. 5).

Serial stereometric measurements were performed only in midshaft and distal shaft segments of straight canals between two adjacent nodes. We started measurement of each internodal segment at a distance of 100 μm from the branches of the node to avoid the funnel effect present in correspondence of all the network nodes. For each paired hemicanal, we first captured an image enlarged $\times 80$ with the electron beam perpendicular to the surface at a working distance of 10 mm; the specimen was tilted first clockwise and then anticlockwise 5° in the plane perpendicular to the main axis of the canal. The corresponding images of the paired hemicanals were captured and processed in the same

Fig. 4 The types of network nodes are shown as they appear on paired fractured surfaces observed with SEM. All the possible combinations of three-arm (3A), four-arm (4A), and five-arm (5A) nodes are presented. The arms with a direction perpendicular to the fracture plane are depicted by ovals. The numbers inside the nodes indicate the arms of each node.

way by the stereometric program [14]. To merge and then stereometrically measure the entire canals, we needed reference points on each of the pair images. For this, we identified morphologic details present on both surfaces, such as the apex of an acute angle bifurcation or osteocytic lacunae (Fig. 5). The two files were merged coupling the reference points, and the corresponding hemicircumferences at the selected locations were calculated. The canal circumference was the sum of the two hemicircumferences. Measurements of hemicircumferences were made at 10- μ m intervals from the reference point (Fig. 5). Artifacts of the canal border could prevent measurement of a hemicircumference along one of the 10- μ m locations, and in this case we ignored that location. The percentage of such missed locations was 3.5% and in no case was the interval between measured locations greater than 20 μ m.

For each canal, the circumference was calculated as the mean of the circumferences at regular intervals along the longitudinal direction, and the mean circumference of Group A and Group B canals were compared for all six rabbits. The canals' circumferences were determined independently by two observers (TC, MR). Intraobserver precision and interobserver precision were expressed as a coefficient of variation of repeated measurements given on

Fig. 5 SEM of paired fractured surfaces shows individual Haversian canals, which are selected arbitrarily (see Figure 3). For each canal, a direction is assigned, corresponding to the advancing direction of the cutting cone if it has been taken in the fracture plane (Group A = yellow) (green arrow), or it is assigned arbitrarily (Group B = blue and red) (green arrows labeled with a). A well-characterized morphologic detail in each canal is selected as the reference point (dot) and hemicircumferences are measured on the mirror-like surfaces at the same distance from it; the sectional planes are selected at regular intervals. We avoided measuring at nodes; supplementary levels of circumference assessment were centered specifically on areas of interest. The colors indicate the corresponding levels of measurement in the same canal on the paired fractured surfaces; yellow illustrates a cutting cone (bar = 500 μ m).

a percentage basis. The former was 7.6% and the latter 9.2%. According to the direction assigned to each canal and to test the hypothesis of convergence of the osteonal lamellar apposition, the difference between two consecutive circumferences (C1 and C2) was indicated as 0 (not significant) when its value was equal to or less than a threshold represented by the mean intraobserver variation plus two standard deviations; otherwise, the difference was designated as positive if $C1 > C2$ or negative if $C1 < C2$.

We compared differences in the density of vascular openings between the endosteal and periosteal surfaces and between subregions (midshaft, middle, and distal shafts) using analysis of variance. The differences in density of Haversian canal branches of the midshaft and distal shaft and those of the mean circumference between Groups A and B canals were determined with Student's *t* test. Intraobserver and interobserver variation for the latter method of measurement was evaluated with the same test. The Kolmogorov-Smirnov test was used to determine if distribution of the variables was normal for each data set. The distribution of node types in the midshaft and distal shaft and the circumference variations in sequential measurements were expressed with the frequency of each node type or circumference variation type (percent of the total number in each class) and analyzed with the Pearson chi square test (nonparametric).

Results

Canals of the endosteal middiaphysis appeared as black spots, rounded or elliptical, from 30 μ m to greater than 1000 μ m in diameter (Fig. 6A). Toward the metaphysis, the surface became rough and irregular; therefore, at this level, more intracortical canals opened inside endosteal crevices or niches (Fig. 6B); often they had the shape of a coil extending into the cortex (Fig. 6C). The periosteal surface showed wide, smooth zones similar to that of the endosteal surface, but the zones alternated with parallel grooves oriented along the longitudinal axis of the femur and with the canal openings uniformly positioned at the same end of the groove (Fig. 7). The density of canal openings was greater ($p = 0.044$) on the endosteal surface at midshaft level (Table 1).

The fracture plane exposed Haversian canals lying in the fracture plane and in shorter convoluted canals. The Haversian canals had a prevailing, straight trajectory but with a succession of straight segments and small deviations at the sites of branching (nodes). Bifurcations with angles between 10° to 30° and 150° to 170° contributed to this geometric figure (Fig. 3). However, other branches had a value of the incidence angle near 90° contributing to the complex, three-dimensional aspect of the internal network.

Fig. 6A–C (A) SEM shows endosteal midshaft canal openings. (B) Distal shaft endosteal canal openings are irregular in size and shape. The vascular canal openings have an ear-like shape. (C) SEM shows endosteal canals of the distal shaft at higher enlargement. The shallow niches scattered on the endosteal surface are artifact resulting from dehydration but correspond to the underlying, more superficial osteocytic lacunae.

There was no difference in the density of the canal network nodes between the midshaft and distal shaft and none in the distribution of three-, four-, and five-arm nodes between the midshaft and distal shaft (Table 2).

The mean circumference of Group A canals (the cutting cones and those that have not yet completed their concentric lamellar system) was greater ($p = 0.000$) than that

Fig. 7 SEM of the periosteal midshaft shows canal openings with grooves; they always are positioned at the same extremity of the groove (toward the midshaft).

Table 1. Density of vascular openings on endosteal and periosteal surfaces

Surface	Number of femurs	Density of vascular openings (number/mm ²)		
		Midshaft	Middle	Distal shaft
Endosteal	6	44.84 ± 19.6	27.93 ± 10.94	27.56 ± 4.41
Periosteal	6	25.86 ± 4.84	24.47 ± 4.5	22.2 ± 5.6
		$p = 0.044$	$p = 0.490$	$p = 0.095$

Values are expressed as mean ± standard deviation.

of Group B (with a small central canal and a complete system of concentric lamellae) (Table 3). The circumference variations of the Group A canals decreased regularly in the direction opposite the advancement of the cutting cone, whereas in Group B, the variations in circumference appeared random relative to the direction of the cutting cone. The percentage of nonsignificant variations between two consecutive circumferences was greater ($p = 0.000$) in Group B canals, whereas the percentage of significant reductions was greater ($p = 0.000$) in Group A (Table 3).

The Haversian canal inner wall was lined by a texture of bundles of collagen fibers densely packed together. The bundles were intertwined along the longitudinal axis of the canals, and holes with a diameter between 0.1 and 0.5 μm were regularly scattered between the bundles. Isolated collagen fibers without a definite orientation were present on the surface of the bundles (Fig. 8A). Corresponding to the canal network nodes, the collagen fiber bundles lined the diverging branches without formation of sharp edges (Fig. 8B). Occasionally, osteocytic lacunae opened on the inner surface of the canal (Fig. 8C). Osteocytic lacunae on the fractured surface were lined by a less densely packed texture of collagen fibers than those of the inner canal wall and had no definite orientation (Fig. 9A). Holes in the same

Table 2. Density and frequency distribution of the different types of nodes

Variable	Number of femurs	Midshaft			Distal shaft			p Value
		3A	4A	5A	3A	4A	5A	
Distribution (%)	6	83.3	14.3	2.4	74.5	18.2	7.3	0.286
Density (number/mm)*	6	4.38 ± 0.89			6.28 ± 3.06			0.175

* Values are expressed as mean ± standard deviation; 3A = three-arms node; 4A = four-arms node; 5A = five-arms node.

Table 3. Canal circumference and circumference variations measured on paired fractured surfaces

Variable	Number of femurs	Group A	Group B	p Value
Canal circumference (µm)	6	176.6 ± 66.3	43.95 ± 9.57	0.000
Circumference variation in sequential measurements (%) [†]	6			
0		32.3	76.6	0.000
+		2.9	13.3	0.000
–		64.8	10.1	0.000
Total		100	100	

* Values are expressed as mean ± standard deviation; [†]indicated as 0 if the difference between two consecutive circumferences (C1 and C2) ≤ the mean intraobserver variation + 2 standard deviations; + if C1 > C2, and – if C1 < C2 and expressed as a percentage of the total number of variations in each class; Group A = canals coming to an end with a cutting head; Group B = canals without a cutting head.

range of sizes (0.1–0.5 µm) were evident between these fibers and corresponded to the openings of canaliculi radiating from the lacunae (Fig. 9B). The head of the cutting cone formed typical resorption pits. The distal shaft paired surfaces included, in addition to a few straight canals, a large number of branched, convoluted, shorter tunnels consistent with the geometry of the periosteal-derived canal system (Fig. 10).

Discussion

The current model of compact bone is that of a system of longitudinal canals (Haversian canals) connected by transverse canals (Volkmann's canals). Models based on histology or microcomputed tomography lack the morphologic detail and sense of temporal development provided by direct observation. Using direct SEM observation, we studied the bone surface and structure of the intracortical canal system in rabbit femurs (1) to determine the density of canal openings on the periosteal and endosteal surfaces, (2) to count and classify the internal network nodes and stereometrically assess canal size, and (3) to describe the collagen fibers bundles lining the inner canals system.

The precision of measurements performed in this study is subject to some bias owing to shrinkage of the specimens secondary to processing, precision required for pairing the mirror-like fractured surfaces, and selection of the

corresponding hemisectional circumferences. However, shrinkage influences absolute measurement but not comparison between different segments of the diaphysis, because it should be the same at both sites. The problems linked to pairing the specular surfaces are part of precision and accuracy of measurements pertaining to the method of study. Our interobserver and intraobserver variability had coefficients of variation of 9.2% and 7.6%, respectively, which allowed the analysis of circumference variations in Groups A and B osteons.

The pairing of the fractured surfaces created a three-dimensional configuration of the canal and made it possible to measure the circumference or the sectional area along its trajectory. Remarkable differences of measurements between two-dimensional and three-dimensional morphometry have been documented with corrosion casting [16, 17]; however, it is difficult to obtain a cast of the intracortical network because of the high resistance to perfusion owing to the viscosity of the resin. Therefore, the mirror-like imprints currently are the only possibility of using three-dimensional stereometry. Moreover, the fracture plane is determined by the applied mechanical forces and by the lines of lower resistance of the mineralized matrix corresponding to canals; therefore, the exposed segment of the canal is much wider than that obtained by any other technique in which the cut plane is rigidly fixed by the sawing device.

The canal openings on the surface reflect distribution of the vessel network inside the cortex and both are dependent

Fig. 8A–C (A) SEM of the internal surface of the Haversian canal shows mineralized collagen fibrils, densely packed in bundles, mostly longitudinally oriented. They intermingle and frequently change direction, leaving spaces for osteocyte canaliculi (arrows). (B) SEM shows the internal surface at a bifurcation of the Haversian canal. The collagen sheath appears partially mineralized and shows disposition of the collagen bundles, which rearrange after branching. A partially formed osteocytic lacuna (arrow) opens on the internal surface of the canal. (C) A SEM enlargement of the osteocytic lacuna in illustration (B) shows the collagen texture in continuity with the canal sheath. The openings of the canaliculi are easily distinguished (arrowheads).

on the extracortical blood circulation. The periosteal vessel network gradually is incorporated by the peripheral apposition and the grooves correspond to the imprints of

Fig. 9A–B (A) SEM shows an osteocytic lacuna exposed by the fracture. The internal surface shows a partially mineralized loose collagen texture. The interwoven fibrils define the openings of the canalicular system (arrowheads). (B) SEM detail shows a canaliculus spreading from the osteocytic lacuna. The conical shape at the origin of the tunnel is evident.

Fig. 10 SEM shows a periosteal-derived canal system in the distal shaft characterized by short, branched tunnels. A larger and straight Haversian canal goes across the fracture surface (asterisk).

the vessels before they seep into the cortex. The well-defined proximal polarization corresponds to the growth vector of the femur. Endosteal holes, on the contrary, are

the entry or exit of canals dug by the osteoclasts. The morphology of distal shaft openings suggests they are larger than those of the midshaft and this is consistent with the general vascular pattern of the bones documented by microradiographic studies [2, 25]. The higher density of canals on the endosteal than on the periosteal midshaft is evidence that new canals are dug to allow new vessels to enter the cortex from the marrow vascular network and that such addition does not occur on the periosteal surface [21, 23]. These findings are in agreement with the actual models of blood flow dynamics in long bones [3, 4, 8, 13, 19, 20, 24, 27].

All current models of the canals' system [5–7, 18, 30–32] rest on the assumption of the Haversian canal as an univocally definite anatomic unit; the higher resolution of the method used here clearly showed the definition of the Haversian canal in its longitudinal extension is arbitrary, even in a theoretical fractured surface, which would extend along the whole diaphysis, and the canal system is more properly represented by a network model consisting of a succession of straight segments with small deviations in correspondence with bifurcations (three-arm node). It is possible to measure the angle of the bifurcations that lie in the fracture plane and to identify two main patterns: acute angles with values of 10° to 30° and 150° to 170° (in relation to the direction of advancement assigned to the specific canal) and others, which usually open on the floor or the roof of the sectioned canal and whose angle cannot be measured; however, the vertical perspective of their shape suggests an angle of approximately 90° . In the advancement of secondary canals, the cutting head usually is formed by more than one osteoclast and the straight direction is the result of an ordered and symmetric distribution of the resorption activity on the front of advancement. When the cells diverge, canal bifurcation is created with the shape of an acute angle node. Right-angle nodes are better explained by the intersection of the cutting cone with a periosteal-derived canal lying in a transverse plane [22, 23]. Resorption spaces with cutting cones at both ends have been reported [6, 31], and this observation could be consistent with the histologically documented canals dug by a cutting cone entering through the endosteal surface and then forming a "T" bifurcation with one proximally and the other distally directed arm [22] (Fig. 11A). More likely are loops with return flow through lateral connections (Fig. 11B). Stereometric measurements of canal circumferences showed the mean circumference was considerably greater in the cutting cones and osteons of Group A; the convergence of canal circumference variations was observed only in the canal segment that immediately followed the cutting head, suggesting, in agreement with other methods of study [11, 12], a faster and steady lamellar apposition in the first phase of osteon

Fig. 11A–B (A) A diagram shows intracortical flow dynamics in resorption spaces with cutting cones at both ends. They correspond to those cutting cones that enter into the cortex through the endosteal surface and then form a "T" bifurcation with two branches, one proximally and the other distally directed. MC = marrow cavity; small arrows = blood flow direction; arrowheads = cutting cone advancing direction; striped circle = canal sectional area. (B) The relationship of intracortical flow dynamics with the size of canals is illustrated. Every advancing cutting cone needs a vascular loop with an afferent vessel and one or more return vessels; this represents a limit to size reduction, at least until the return flow is deviated in a collateral canal (striped circle = actual canal sectional area; dotted circle = earlier canal sectional area).

Fig. 12A–B The diagrams show two possible models of osteon reduction: (A) segmental and more irregular distribution of lamellar apposition and (B) regular progression of concentric lamellar apposition as far as the central canal reaches the definitive size of the completely structured osteon. Measurements performed in this study support Model A.

formation (Group A). In advanced structured osteons (Group B), circumference convergence no longer was observed, as would be expected by regular distribution of the concentric lamellar apposition along the advancing osteon (Fig. 12).

Study of the collagen fiber pattern lining the inner surface of the canals documented extensive connections with the canalicular system through small pores between the collagen bundles, not different from those present on the inner surface of the osteocytic lacunae. The size and shape of the osteon central canal are determined by two factors: the original tunnel dug by the osteoclasts and the lamellar apposition. Whether the size variations of the central canal are determined from the origin by an irregular shape of the tunnel or by a discontinuous apposition cannot be tested with the methods we used. However, in the early phase of tunnel development, it must be sufficiently wide to contain the vascular loop of the cutting cone. These vessels do not fill completely the canal lumen, but when the lamellar apposition advances they become a mechanical obstacle to additional reduction of the canal sectional area. Therefore osteon morphology cannot be explained exclusively by the role of control and regulation of the osteoblast/osteocyte mechanism on bone apposition [1, 15], but also by consideration of the blood flow dynamics because the completely structured osteons as a rule have a single capillary-like vessel filling the whole space of the central canal.

We believe our data improve the knowledge of the bone organization and provide the basis for the study of aging and involution of the cortical bone in osteoporosis and other diseases characterized by bone loss.

Acknowledgments This research was performed with a high-resolution scanning electron microscope of the Centre for Large Instruments for Biomedical Research at the University of Insubria. We thank Dr Michele Gneccchi for valuable help with statistics.

References

1. Bell KL, Loveridge N, Reeve J, Thomas CD, Feik SA, Clement JG. Super-osteons (remodeling clusters) in the cortex of the femoral shaft: influence of age and gender. *Anat Rec*. 2001;264:378–386.
2. Brookes M, Landon DN. The juxta-epiphyseal vessels in the long bones of foetal rats. *J Bone Joint Surg Br*. 1964;46:336–345.
3. Brookes M, Lloyd EG. Marrow vascularization and estrogen-induced endosteal bone formation in mice. *J Anat*. 1961;95:220–228.
4. Brookes M, Revell WJ. *Blood Supply of Bone: Scientific Aspects*. London, UK: Springer Verlag; 1998.
5. Cohen J, Harris WH. The three-dimensional anatomy of Haversian systems. *J Bone Joint Surg Am*. 1958;40:419–434.
6. Cooper DM, Thomas CD, Clement JG, Hallgrímsson B. Three-dimensional microcomputed tomography imaging of basic multicellular unit-related resorption spaces in human cortical bone. *Anat Rec A Discov Mol Cell Evol Biol*. 2006;288:806–816.
7. Cooper DM, Turinsky AL, Sensen CW, Hallgrímsson B. Quantitative 3D analysis of the canal network in cortical bone by micro-computed tomography. *Anat Rec B New Anat*. 2003;274:169–179.
8. Draenert K, Draenert Y. The vascular system of bone marrow. *Scan Electron Microsc*. 1980;4:113–122.
9. Hert J, Fiala P, Petryl M. Osteon orientation of the diaphysis of the long bones in man. *Bone*. 1994;15:269–277.
10. Hert J, Hladíková J. The vascular supply of the Haversian bones. *Acta Anat*. 1961;45:344–361.
11. Jaworsky ZF, Duck B, Sekaly G. Kinetics of osteoclasts and their nuclei in evolving secondary Haversian systems. *J Anat*. 1981;133:397–405.
12. Jaworsky ZF, Hooper C. Study of cell kinetics within evolving secondary Haversian systems. *J Anat*. 1980;131:91–102.
13. Lopez-Curto JA, Bassingthwaite JB, Kelly PJ. Anatomy of the microvasculature of the tibial diaphysis of the adult dog. *J Bone Joint Surg Am*. 1980;62:1362–1369.
14. Manelli A, Sangiorgi S, Binaghi E, Raspanti M. 3D analysis of SEM images of corrosion casting using adaptive stereo matching. *Microsc Res Tech*. 2007;70:350–354.
15. Metz LN, Martin RB, Turner AS. Histomorphometric analysis of the effects of osteocyte density on osteonal morphology and remodelling. *Bone*. 2003;33:753–759.
16. Minnich B, Lametschwandner A. Lengths measurements in microvascular corrosion castings: two-dimensional versus three-dimensional morphometry. *Scanning*. 2000;22:173–177.
17. Minnich B, Leeb H, Bernroider EW, Lametschwandner A. Three-dimensional morphometry in scanning electron microscopy: a technique for accurate dimensional and angular measurements of microstructures using stereopaired digitized images and digital image analysis. *J Microscopy*. 1999;195:23–33.
18. Mohsin S, Taylor D, Lee TC. Three dimensional reconstruction of Haversian systems in ovine compact bone. *Eur J Morphol*. 2002;40:309–315.
19. Morgan JD. Blood supply of growing rabbit's tibia. *J Bone Joint Surg Br*. 1959;41:185–203.
20. Ohtani O, Gannon B, Ohtsuka A, Murakami T. The microvasculature of bone and especially of bone marrow as studied by scanning electron microscopy of vascular casts: a review. *Scan Electron Microsc*. 1982;(Pt 1):427–434.
21. Pazzaglia UE, Andriani L, Di Nucci A. The reaction to nailing or cementing of the femur in rats: a microangiographic and fluorescence study. *Int Orthop*. 1997;21:267–273.
22. Pazzaglia UE, Bonaspetti G, Ranchetti F, Bettinsoli P. A model of the intracortical vascular system of long bones and of its organization: an experimental study in rabbit femur and tibia. *J Anat*. 2008;213:183–193.
23. Pazzaglia UE, Bonaspetti G, Rodella LF, Ranchetti F, Azzola F. Design morphometry and development of the secondary osteonal system in the femoral shaft of the rabbit. *J Anat*. 2007;211:303–312.
24. Rhinelander FW, Stewart CL, Wilson JW. Bone vascular supply. In: Simmons DJ, Kunin AS, eds. *Skeletal Research: An Experimental Approach*. New York, NY: Academic Press; 1979:367–395.
25. Rogers WH, Gladstone H. Vascular foramina and arterial supply of the distal end of the femur. *J Bone Joint Surg Am*. 1950;32:867–874.
26. Shenk R, Willenegger H. On the histology of primary bone healing. *Langenbecks Arch Klin Chir Ver Dtsch Z Chir*. 1964;308:955–968.
27. Skawina A, Litwin JA, Gorczyca J, Miodonski AJ. The vascular system of human fetal long bones: a scanning electron microscope study of corrosion casts. *J Anat*. 1994;185:369–376.
28. Smith JW. Collagen fibre patterns in mammalian bone. *J Anat*. 1960;94:329–344.
29. Smith JW, Andrew S. Age changes in the organic fraction of bone. *J Bone Joint Surg Br*. 1963;45:761–769.

30. Stout SD, Brunsden BS, Hildebolt CF, Commean PK, Smith KE, Tappen NC. Computer-assisted 3D reconstruction of serial sections of cortical bone to determine the 3D structure of osteons. *Calcif Tissue Int.* 1999;65:280–284.
31. Tappen NC. Three-dimensional studies of resorption spaces and developing osteons. *Am J Anat.* 1977;149:301–332.
32. Thompson JC. *Netter's Concise Atlas of Orthopaedic Anatomy.* Teterboro, NY: Multimedia USA Inc; 2002.