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3D imaging of the lacunar-canalicular network of compact bone

T. Congiu[1], U.E. Pazzaglia[2], P. Basso[1], M. Raspanti[1]

[1]Insubria University, Dept. Surgical & Morphological Sciences, Varese, Italy
[2]Brescia University, Dept. Surgical Specialties, Brescia, Italy

mario.raspanti@uninsubria.it
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The canalicular network which interconnects osteocytic lacunae in compact bone and which maintains the osteocytes in reciprocal contact is a critical pathway for the supply of fluid and nutrients that keep the bone cells alive and functional. In addition it has been hypothesized that the flow of fluids and substances through the lacunar-canalicular network may be a necessary element for the regulation of the bony cells activity and the balance between bone resorption and deposition.

The intricate meshwork of tiny passages has been the subject of a few studies, always carried out by sectional techniques – either by serial sections of embedded specimens, or by confocal laser scanning microscopy, or by FIB/SEM imaging. All these techniques can only visualize a limited volume of the bone matrix, and in all cases the persistence of the dense bone matrix represents a major obstacle to the visualization of the finer details.

In the present research the lacunar-canalicular meshwork of the compact bone has been studied by a variation of the corrosion casting technique, an approach which so far was rarely used [1,2]. Small fragments of bone compact from the diaphysis of human tibiae were thoroughly cleaned in hydrogen peroxide in order to get rid of the cells, the blood vessels and the non-mineralized tissues in general. Two different acrylic resin were then used to obtain a detailed cast of the bone cavities, that has been subsequently visualized by scanning electron microscopy (SEM).

In a first case the clean bone block was infiltrated with a biocompatible polymethylmetacrylate (PMMA) resin, usually used as an orthopaedic cement (DePuy International, Blackpool, UK). The infiltration of the monomer in the Havers canals and hence in the lacunar-canalicular network was simply driven by capillarity, helped by the evaporation. In a successive variant a different PMMA formulation (Heraeus Kulzer, Hanau, Germany) was used. In this case, being the monomer preparation more viscous, the infiltration was helped by high pressure (approx. 700 kPa).

The specimen was then etched in HCl and then in KOH solution to completely dissolve the bone matrix, freeing the cast of the canalicular mesh and allowing its observation by SEM (Figure 1). The cast duplicated faithfully the shape, position and connection of the osteocytic lacunae and afforded an unrestricted exploration of their spatial relations without any other manipulation.

Our observation revealed the existence of two distinct systems of canalicula: in addition to a radial canalicular system which interconnects the osteocytes of successive lamellae there is indeed an equatorial system of canalicula which interconnect the osteocytes of the same plane (Figure 2). Together, these two groups form a really three-dimensional network.

A morphometric study of the length and width of the osteocytic interconnection is still under way. Our results, however, already suggest that a fluids flow trough the canalicular network is not necessary, and that the supply of oxygen and metabolites from the vascular canals to the most peripheral osteocytes could be assured simply by cell-to-cell diffusion through the canalicular network.

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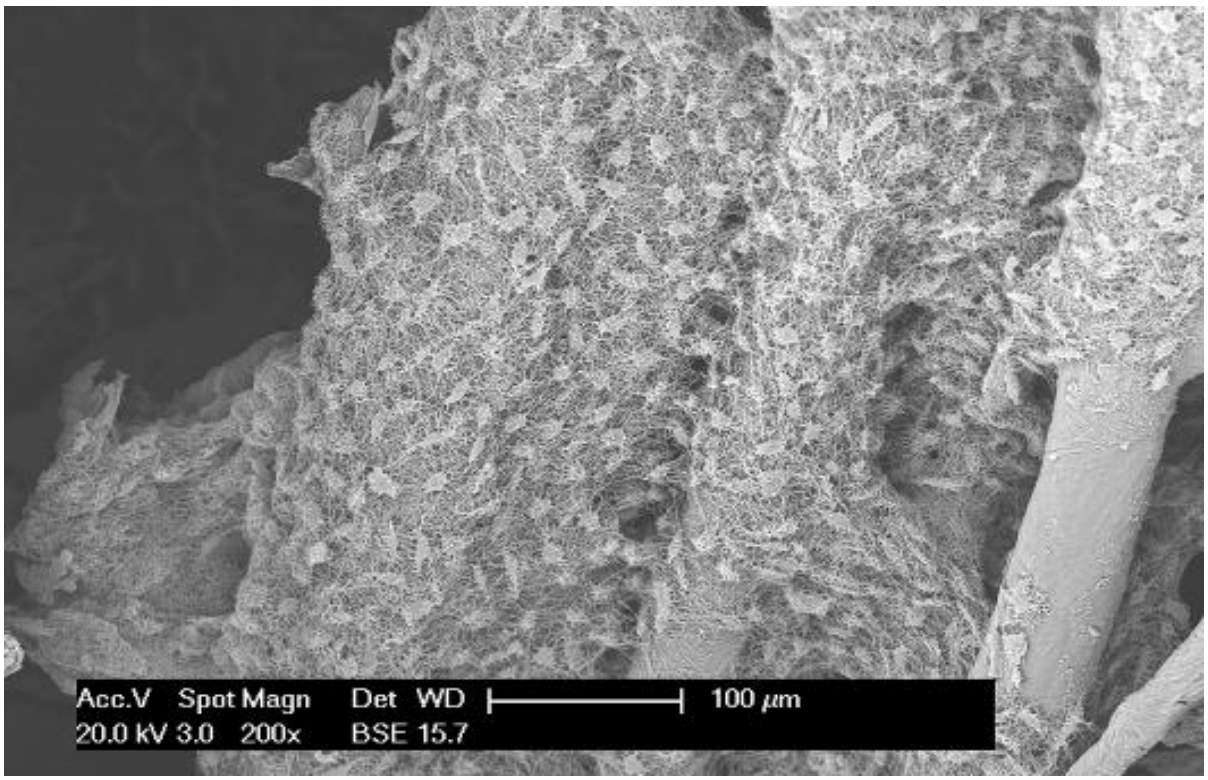

Figure 1. Low-magnification of the lacunar-canalicular network around the Haversian canals.

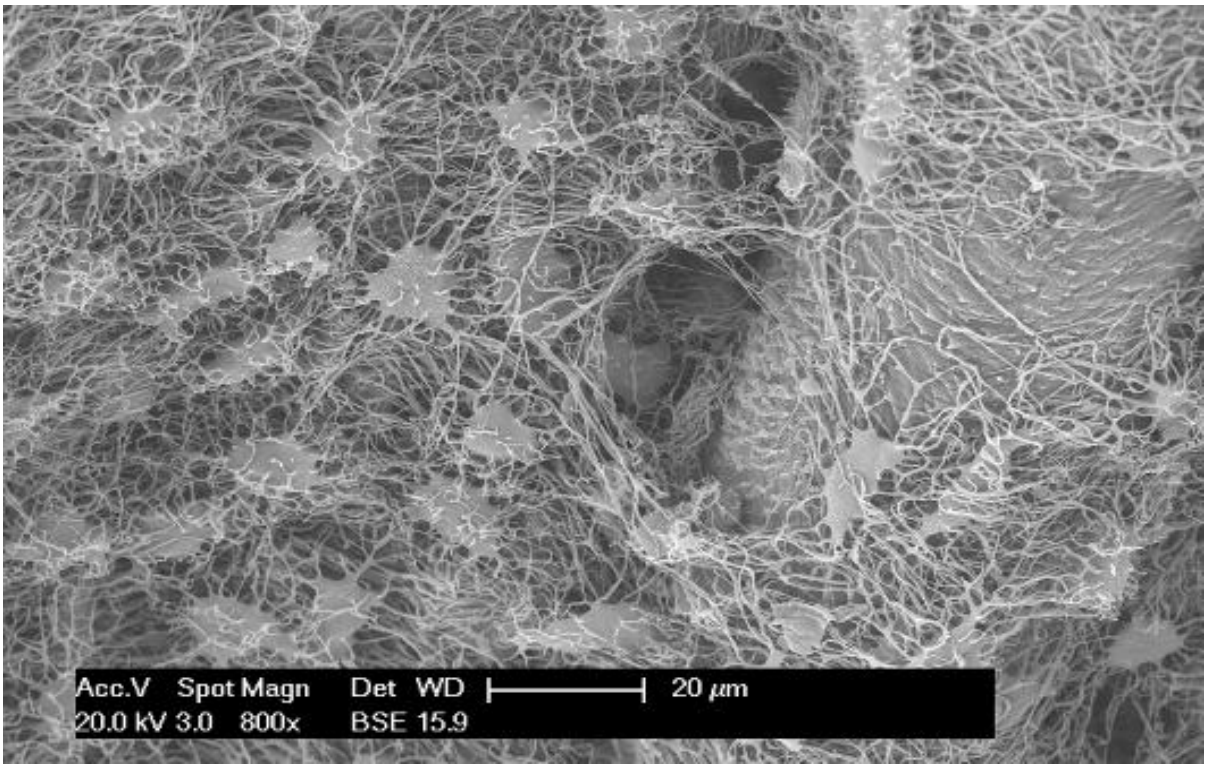

Figure 2. A higher magnification reveals the emergence of the canalicula not only from the osteocytes radial surfaces but from their edge as well.