

3D imaging of the osteon lacunar-canalicular system: an improved corrosion-casting method

Terenzio Congiu¹, Marina Protasoni¹, Mario Raspanti¹ and Ugo Ernesto Pazzaglia²

¹Department of Surgical & Morphological Sciences, Insubria University, Varese, Italy

²Department of Surgical, Radiological & Medical Specialties, Brescia University, Brescia, Italy

The osteocyte lacunar-canalicular system has been the subject of several studies either by serial sectioning of embedded specimens, or by confocal laser scanning microscopy, or with FIB/SEM imaging. With all these techniques the persistence of the bone matrix hampers the visualization of the finer details of the canalicular network. In the present research the methyl-metacrylate casting technique was applied to human cortical bone in order to evidenciate the 3-D organization of the osteons lacunar-canalicular system. The MMA monomer infiltration into the vascular canals, and hence into the lacunar-canalicular system was driven by capillarity, helped by evaporation and the resulting negative pressure. The resin penetration was complete in the Haversian canal but limited to a depth of 4 – 5 layers of lacunae around the canal itself, suggesting that each secondary osteon is a close system limited by an impenetrable cementing line. The casts duplicated the shape, position and connections of the lacunae, and allowed an unrestricted exploration of lacunar-canalicular network without manipulations such as cutting or sawing. Two systems of canalicula could be distinguished: the equatorial, which connects osteocytes lying on the same concentric level, and the radial, which interconnects different levels. The equatorial canalicula radiate from the lacunar border on a planar surface around the lacuna, forming straight and basically parallel canals; the radial have a predominant direction perpendicular to the equatorial plane. The mean length of the radial canalicula was $38.4 \pm 7.35 \mu\text{m}$ in human osteons, while their mean diameter was $195.7 \pm 79.58 \text{ nm}$. The equatorial canalicula had an average diameter of $249.7 \pm 73.78 \text{ nm}$, significantly larger ($p < 0.001$) than the radial. The architecture of lacunar-canalicular network obtained by the MMA casts was compared with theoretical models. Our data suggests that the intracanalicular pericellular fluid is static and that the supply of ions, oxygen and metabolites to the osteocytes can be assured by the diffusion of these molecules into the static volume of the solution without the need to hypothesize a fluid flow within the canalicular system.

Keywords: Bone, osteon, corrosion casting, SEM.