Original Article

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Anatomy Section

Osteocyte Lacunae are Lenticular/Ellipsoid Spaces or Spiral/Helical Tubules

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ABSTRACT

Introduction: According to the current concept of bone structure, the osteocyte lacunae are lenticular or ellipsoid spaces occupied by osteocytes. These osteocytes are thought to communicate with each other through a tubular system made up of Canaliculi. The rest of the structures i.e., Haversian canal, and Volkmann's canal are also tubular in shape. Considering this existing concept of bone microstructure described by various authors, it is highly unlikely that the lacunae alone would be lenticular/ellipsoid structure. In the present study author wanted to know that amongst the all tubular spaces, what is the reason that only the osteocyte lacunae are lenticular or ellipsoid structure? Also to investigate whether these lenticular spaces are really lenticular/ellipsoid or they are cut sections of tubes, which are lying helically or spirally. It is well known from various previous studies that Haversian canal, Volkmann's canal and Canaliculi are tubular shaped structures and how it is possible that lenticular/ellipsoid structure can be present among them. So, it may be possible that these lenticular spaces are not actually lenticular but this lenticular shape is due to the cut sections of any type of tubule in various possible planes (i.e., transverse, longitudinal and various degrees of oblique plane).

Aim: The present study was carried out to reinvestigate the shape of the osteocyte lacunae amongst the tubular system (i.e., Haversian canal, Volkmann's canal and Canaliculi) of compact bone.

Materials and Methods: The study is carried out by preparing thin sections of adult bones (ground glass preparation) and visualizing them under binocular light microscope and scan-electron microscope after following proper procedure.

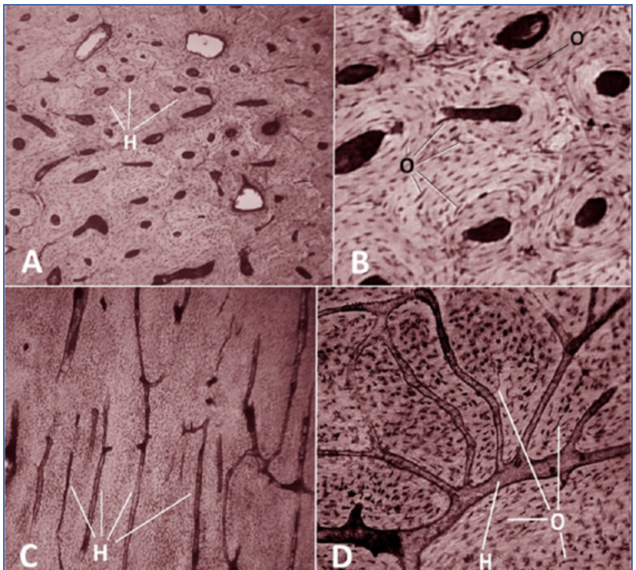
Results: We observed that the lacunae are actually spirally/helically placed tubules with several branching. These branching are considered as canaliculi. These branching are of various diameters and they anastomose between themselves. It does appear that the so called osteocyte lacunae are optical illusions in the light microscope representing the depth of the lumen of these tubular spaces, which get sectioned in all possible planes.

Conclusion: From the present study we concluded that the whole structure of mature bone is tubular in shape without the presence of lenticular/ellipsoid lacunae. The transport of organic and inorganic material may be by free flow mechanism through this tubular framework. We hope that this fresh understanding of basic bone structure will open new vistas for further research.

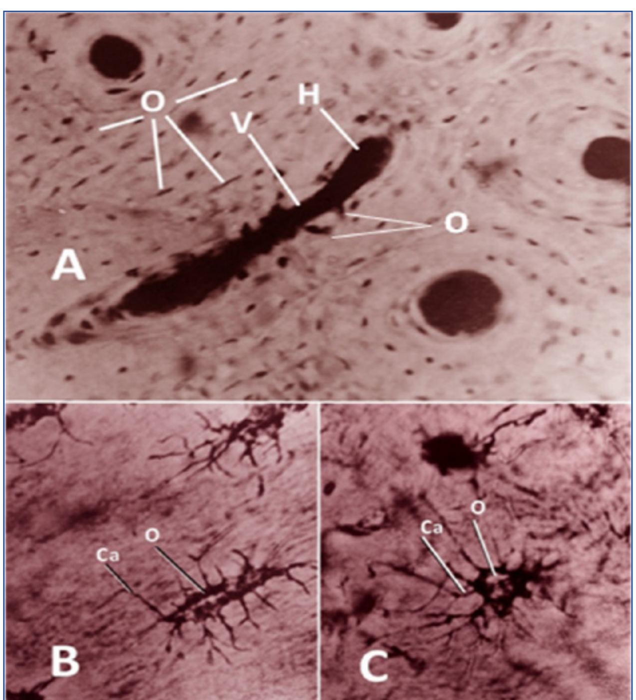
Keywords: Canaliculi, Haversian canal, Lamellae, Volkmann's Canal

INTRODUCTION

The existing concept of the osteocyte lacunae is that they are lenticular spaces concentrically placed along with the lamellae surrounding the Haversian canals. Their long axis is in line with the lamellae [1,2]. From these lenticular spaces radially arranged channels (canaliculi) spread out into the bone matrix and join channels of other lacunae. The lacunae contain osteocytes which are the basic cells of mature bone and responsible for the maintenance of bony structure. These osteocytes send cytoplasmic extensions (dendrites) into the canaliculi and thereby, gain communication with adjacent osteocytes [3,4]. The present study undertaken after a preliminary observation revealed that the lacunae are not just "lenticular spaces" but a part of fine tubular system arising from the well recognized Haversian canals and Volkmann's canals and canaliculi are arising from these tubules [Table/Fig-1,2] [5-7]. The importance of the present study is that it gives information about microstructure of bone and provides anatomical basis of the fact that bones are hard but not brittle, because helical/spiral arrangement provides resilience to the bone [8,9]. Also,



[Table/Fig-1a-d]: Ground preparation of long bone.
1a) Transverse section of Femur seen in low magnification.
1b) Transverse section of Femur seen in high magnification.
1c) Longitudinal section of Humerus seen in low magnification.
1d) Sagittal section of Malleus seen in high magnification.
*(H-Haversian canal, O-Osteocytic tubule, V-Volkmann's canal).



[Table/Fig-2a-c]: Ground preparation of bone in 100 X magnification.
*(H-Haversian canal, V-Volkmann's canal, O-Osteocytic tubule, Ca-Canaliculi).

the study helps in understanding of successful bone grafting which is possible only because of presence of tubular system in the bone (evidenced by the fact that after implanting pieces of bones to the bone of other body part, the tubular pattern of existing bone and tubular pattern of implanted pieces of bone get anastomose and continue with each other. This continuity of tubular pattern is not possible after bone graft if we accept lenticular pattern.

MATERIALS AND METHODS

This observational study was started in 1987 and continues till date. Study was conducted at NSCB Medical College, Jabalpur, Madhya Pradesh and AIIMS, Delhi, India.

We have prepared 3-4 sections (2-3 mm thick) of each plane i.e., sagittal, coronal, transverse and various degrees of oblique sections of shaft of 50 femora, 10 tibiae, 10 humerii, and 10 radiae of adult cadavers were prepared with the help of saw. Total 10 malleus were also used along with these sections. Then above sections and malleus were ground delicately on a special velvet finish French hone to make them extremely thin so that the sections became transparent. The total ground preparations were 1050 in number. Out of these ground preparations 950 were kept on glass slides and dehydrated in alcohol. Then they were mounted on slides using cidar wood oil. These preparations were seen under light microscope (Model - binocular Motic B1 series) in low magnifications and then visualized under oil immersion lens (magnification 100X). The photographs of each preparation were taken. The remaining 100 sections were directly visualized by scan-electron microscope (model-Philips 501 B) after routine preparation that includes chemical fixation of the specimen followed by dehydration in acetone series and then dried at the critical point. Then samples were mounted on a stub of metal with adhesive, coated with 40-60 nm of silver and then observed in the scan electron microscope and photographs were taken.

RESULTS

[Table/Fig-3] shows the number and type of sections in bones studied. [Table/Fig-4] shows findings in low and high magnifications.

Light microscopy: Both Haversian canals and Volkmann's canal were well demonstrated. Concentrically arranged round, oval, oblong or lenticular areas (so called osteocyte lacunae) were seen around the Haversian canals [Table/Fig-1b]. In several sections the so called osteocyte lacunae appeared to communicate with each other and also with Haversian canal/ Volkmann's canals [Table/Fig.-1b]. In some areas a clear tubular structure could be visualized [Table/Fig-1b and d].

Under higher magnification the above mentioned so called osteocyte lacunae were better visualized. Even in the single section they show round, oval, oblong or lenticular shapes [Table/Fig-2b and c]. The whole picture appears to be one of spirally/helically placed tubules which have been sectioned in all possible planes resulting in these areas appearing to be either round, oval, oblong or lenticular. The "areas" themselves

Name of Bone	No.	Sections				Total Sections
		Transverse (3 Each)	Coronal (3 Each)	Sagittal (3 Each)	Oblique (4 Each)	
Femur	50	150	150	150	200	650
Tibia	10	30	30	30	40	130
Humerus	10	30	30	30	40	130
Radius	10	30	30	30	40	130
Malleus*	10	-	-	10	-	10
Grand Total		240	240	250	320	1050

[Table/Fig-3]: Showing number and type of sections in bones studied.

*Only one ground glass preparation could be prepared from each malleus.

		Low Magnification	High Magnification
Light Microscopy (950 Sections)	Transverse Sections (240)	HC and VC were well visualized. Concentrically arranged round, oval, lenticular areas were seen. Communications were also present at some places.	Concentrically placed areas, Round, oval, lenticular and tubular structures were better visualized.
	Longitudinal Sections-Coronal and Sagittal (240+250)	Longitudinally placed tubes were present. Branching and anastomosis were also seen.	Branching and anastomosis of longitudinally placed tubes is well visualized.
	Oblique Sections (320)	A clear tubular structure could be seen.	
Scan Electron Microscopy (100 Sections)		Around Haversian canal incomplete concentric rings were present. Mouth of tubular channels was also seen in HC. Lamellae were also seen as tubular structure.	

[Table/Fig-4]: Showing findings in low and high magnifications.

*(HC-Haversian canal, VC-Volksmann's canal).

represent the lumen of these tubules.

Scan-Electron Microscopy: There is a marked difference in the picture seen in scan electron microscopy from that seen in light microscopy [Table/Fig-5]. The so called lamellae (as mentioned by various authors) seem to be not complete concentric rings placed around the central Haversian canal in transverse sections. The same lamellae may be thicker or thinner along its course and occasionally branched at places [Table/Fig-5a]. In line with the lamellae or sometimes in between two adjacent lamellae we can see round, oval, oblong or lenticular openings. These openings in higher magnification seem to be the mouth of tubular channels [Table/Fig-5c] which in some sections are seen to communicate with finer channels within the lamellae. The lamellae themselves seem to be tubular structures [Table/Fig-5b].

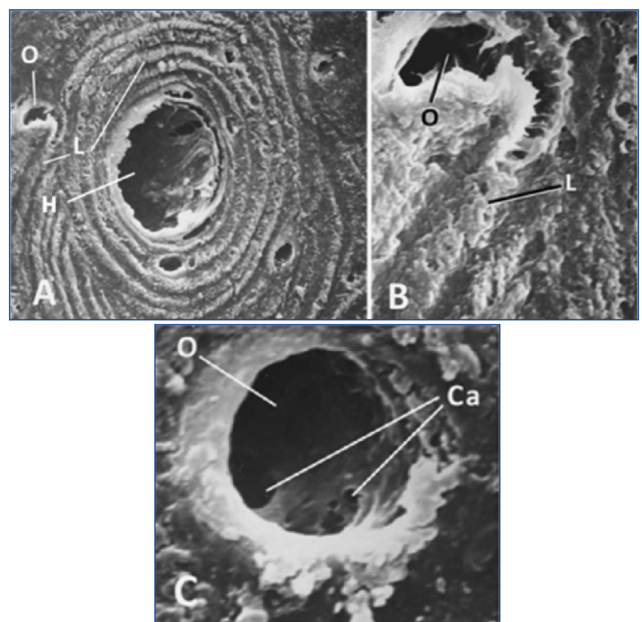
In the interstitial lamellae also tubular structures were seen to cross multiple lamellae. The channels within the lamellae can be demonstrated to open up within these tubules. No osteocyte or other cells were seen in any of the round/ oval/ oblong or lenticular openings (may be due to flushing of these cells during perpetration).

DISCUSSION

The current understanding of the microscopic structure of mature bone revolves around four structural details [10-12]:

1. A system of longitudinally/transversely/obliquely placed tubes-the Haversian and Volkmann's canals.

2. Tubular structures coming out spirally/helically from Haversian and Volkmann's canal-osteocytic tubules.

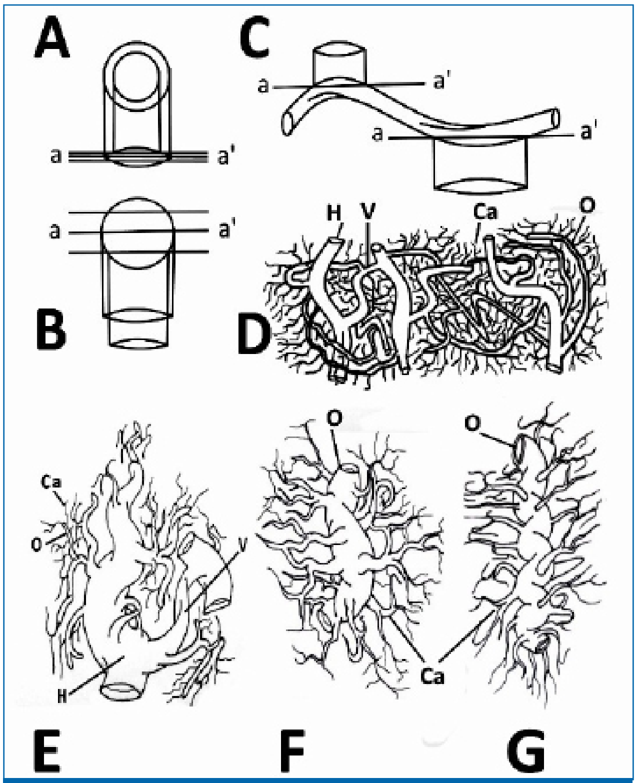


[Table/Fig-5]: Scan electron photomicrograph.
5a) Typical Haversian system.
5b) One osteocytic tubule.
5c) Transversely cut osteocytic tubule in higher magnification. Opening of canaliculi (Ca) are seen from the wall of tubule.
Scale-1 bar=1μ
*(H-Haversian system, L-Lamella, O-Osteocytic tubule, Ca-Canaliculi).

3. Tubular structures coming out from osteocytic tubules-tubules of Lamella (e).

4. Fine tubules (canaliculi) arising mostly from osteocytic tubules as well as from other tubes.

In a tissue where the dominant feature is the presence of a system of tubes, the presence of concentrically placed small spaces and cylindrical lamellae appears very unconvincing



[Table/Fig-6]: Schematic representation of Haversian system.
6a) Lenticular space is cut in aa' axis. We will get round shapes of various diameters.
6b) When lenticular space is cut in aa' axis, we will get spindle shapes of various diameters. The maximum diameter of these shapes is 25 µ.
6c) When a spiral tube is cut, we get spindle shapes of various diameters. The spindle shaped spaces may be more than 25 µ.
6d-g) After the detail analysis of the observations, a schematic plan of tubules has been drawn.

[13-15]. From basic preliminary investigations on light microscopy, we could see that the concentrically placed open areas around the Haversian canals were not only lenticular but also round, oval or oblong in shape. If we consider these so called spaces to be lenticular closed areas only, then the cut section would result in a picture as shown in [Table/Fig-6]. From the sections it is clear that at least some of the round spaces should have similar dimensions as the length of the lenticular spaces. However, the diameter of the round spaces seen did not reach to even half the length (maximum) of the lenticular spaces i.e., about 25 µ. On the other hand the transverse diameter of the oval/lenticular spaces was always less or equal to the diameter of round spaces.

To explain this, we created a spiral/helical tubular model, which we cut in many planes [Table/Fig-6] From this it is easy to explain that the concentrically placed areas around Haversian canals are not closed lenticular spaces but lumen of spiral/helical tubes. On further magnification also, these spaces were seen to be the mouth of the tubes [Table/Fig-5a-c]. We can therefore say that the description of osteocyte lacunae as closed lenticular spaces is misleading.

In our scan electron microscopic observations the lamellae were also seen to be tubular in nature communicating with the tubular network mentioned above [Table/Fig-5]. These tubulations are not simple but sacculated. The finding that they are incomplete circles around the Haversian canals proves that they are not concentric but spirally arranged around the Haversian canals [16,17].

Thus, the whole structure of mature bone appears to be a system of-

1. Tubes-Haversian canals / Volkmann's Canals

2. Tubules-(a) Primary osteocytic tubule arising from both type of tubes (so called osteocyte lacunae); (b) Secondary osteocytic tubules arising from primary tubules (forming lamellae)

3. Canaliculi-arising from tubules and rarely from both type of tubes [Table/Fig-3b,c].

In scan electron microscope photograph [Table/Fig-5b,c] we can see the depth of so called osteocyte lacunae that proves that these structures are tubular in shape. (We cannot see the depth in such magnitude if we accept the lenticular pattern of osteocyte lacunae).

The transport of materials through this system could be by free flow mechanism, for the maintenance of structure of bone. As per previous literature, fluid flows from one osteocyte lacuna to other through canaliculi (smaller in diameter than osteocytic tubule). So there is no free flow of fluid. But, if we accept tubular pattern, then there will be free flow of fluid through spirally/helically arranged osteocytic tubules for maintenance of structure of bone [18].

S.NO.	Author (Years)	Findings
1	Grey's Anatomy (2016) [2]	Ellipsoid in shape.
2	Dong P et al., (2014) [15]	Ellipsoid in shape. (18.9 ± 4.9 X 9.2 ± 2.1 X 4.8 ± 1.1 micron)
3	McCreadie BR et al., (2004) [19]	A large range of size and shape
4	Langer M et al., (2012) [20]	Flattened lenticular drop shape
5	Carter Y et al., (2013) [21]	More elongated and flattened lacunae
6	Van Oers RF et al., (2015) [22]	There is considerable variation in the shape. It may be scalene, prolate, oblate or spherical.
7	Present study	Tubular in shape.

[Table/Fig-7]: Comparison of shape of osteocyte lacunae in present study with previous studies.

Comparison of shape of osteocyte lacunae in present study with previous studies has been presented in [Table/Fig-7].

LIMITATION

The entire path of any tube/tubule is not visible in a single section.

CONCLUSION

The present study concluded that the osteocyte lacunae are in fact, the lumen of tubules around the Haversian canals. The lamellae are also tubular in nature and placed spirally around Haversian canals and communicate with the above mentioned tubes. The authors conclude that the whole structure of mature bone including osteocyte lacunae is one of branching tubes, tubules and microtubules (canaliculi). This spiral/helical pattern of osteocyte tubules provide functional relevance for resilience of the bone and the free flow of fluid. Further detailed studies involving more number and type of bones from various regions are required for the better understanding of bones.

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