The complex channel networks of bone structure

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Bone structure in mammals involves a complex network of channels (Havers and Volkmann channels) required to nourish the bone marrow cells. This work describes how three-dimensional reconstructions of such systems can be obtained and represented in terms of complex networks. Three important findings are reported: (i) the fact that the channel branching density resembles a power law implies the existence of distribution hubs; (ii) the conditional node degree density indicates a clear tendency of connection between nodes with degrees 2 and 4; and (iii) the application of the recently introduced concept of hierarchical clustering coefficient allows the identification of typical scales of channel redistribution. A series of important biological insights is drawn and discussed.

Containing two types of channels, namely Havers and Volkmann channels, the bones of mammals are pervaded by a network of intercommunicating channels required to maintain the bone living cells \( \text{\textsuperscript{1}} \), which is done by blood vessels distributed inside those channels. Although playing a decisive role in animal development, diseases, and bone reconstitution, the topology of the bone channel network remains a subject of investigation. Because of its generality, the concept of complex networks \( \text{\textsuperscript{2}} \) stands out as a natural and comprehensive means to represent, characterize and model the bone channel system. By associating the branching points of channels to the nodes of the network, and representing the connections between such spots in terms of arcs between the network nodes, it is possible to represent the bone channel structure in terms of a complex network. Topological measurements obtained from such networks — especially the node degree density \( \text{\textsuperscript{3}} \), the conditional node density \( \text{\textsuperscript{4}} \), and the hierarchical clustering coefficient \( \text{\textsuperscript{5}} \) — can then be used to identify important underlying features of the analyzed channel system.

The bone used as a biological model in this study is the humerus of the adult cat, collected from necropsy cats in the Sector of Pathology at the Veterinary Hospital of the Federal University of Uberlândia, Brazil. After dissection of the skin and muscles, a ring 0.5 cm wide was removed from the central portion of both right and left humerus of each animal. Such rings were fixed in 10\% formalin for at least 48 hours. After setting, these samples were decalcified in 4\% nitric acid per 30 days, embedded in paraffin according to a classic histological technique, and sectioned in microtome in order to obtain 200 serial sections with 5 \( \mu \text{m} \) thickness. These sections were then stained by the Schmorl procedure, and digital images were acquired by using an Olympus Triocular BX40 microscope connected to an Oly-200 camera interfaced to a PC-compatible computer through digital plate Datatranslation 3135. After the digitalization procedure, we extracted the regions of interest through the segmentation \( \text{\textsuperscript{6}} \) of both Havers and Volkmann channels.

The images with final size of approximately 700\( \times \)480 pixels were converted into PNG format and used to obtain a three-dimensional reconstruction of the studied structure, which was performed by using the vtkPN- GReader class in the Visualization Toolkit for C++. Figure 1(a) shows the three-dimensional reconstruction \( \text{\textsuperscript{7}} \) of part of the analyzed bone structure (one fourth). The detection of nodes of the graph and respective connections was performed manually, yielding an unweighted, non-oriented complex network, which was represented in terms of its \( N \times N \) adjacency matrix \( K \), such that the existence of a connection between any two nodes \( i \) and \( j \) is represented by making \( K(i, j) = K(j, i) = 1 \). The vertices of “V”-like channels were understood to correspond to branching spots with missing connections, giving rise to nodes with degree 2. Figure 1(b) shows the geographic complex network (the nodes have positions in \( R^2 \) ) obtained from the structure in Figure 1(a) by projecting the node coordinates along the \( z \)— axis. The degree of a node \( i \) is defined as the number of edges connected to that node, which can be calculated as \( k(i) = \sum_{q=1}^{N} K(i, q) \). Figure 2(a) shows the dilog plot of the node degree density obtained for the bone considered in the present study, which is characterized by 988 nodes and 1120 links.

The obtained density profile indicates that the bone channel network resembles a scale free model \( \text{\textsuperscript{8}} \), which is characterized by the existence of hubs, i.e. nodes with particularly high degrees which act as concentrators of connections. Such a finding rules out the possibility that the channel system could be organized as a regular network or a tree, both characterized by similar node degrees throughout. The conditional node density of the bone channel structure is shown in Figure 2(b). It is

\[ K(i, j) = 1 \]

\[ k(i) = \sum_{q=1}^{N} K(i, q) \]

\[ K(i, j) = K(j, i) = 1 \]

\[ K(i, q) \]

\[ k(i) \]

\[ \sum_{q=1}^{N} K(i, q) \]

\[ \text{\textsuperscript{1}} \]

\[ \text{\textsuperscript{2}} \]

\[ \text{\textsuperscript{3}} \]

\[ \text{\textsuperscript{4}} \]

\[ \text{\textsuperscript{5}} \]

\[ \text{\textsuperscript{6}} \]

\[ \text{\textsuperscript{7}} \]

\[ \text{\textsuperscript{8}} \]
clear from such a density that nodes with degree 2 tend to connect with nodes with degree 4, which may be related to the fact that the circulation of the arteries inside the channels follows an Eulerian network, which is characterized by nodes with even degree [12].

In order to further understand the topological structure of the channel system, we considered the recently introduced concept of hierarchical clustering coefficient. Take a generic node in the network, represented by \(i\). Let \(H(i, d)\) be the set of nodes which are at distance \(d\) from node \(i\) (the distance between two nodes \(i\) and \(j\) corresponds to the number of edges along the shortest path between those nodes). Observe that the consideration of subsequent values of \(d\) defines a hierarchy around the reference node \(i\). Now, the hierarchical clustering coefficient of node \(i\) at distance \(d\) can be defined as follows

\[
HCC(i, d) = \frac{E(H(i, d))}{||H(i, d)|| \left( ||H(i, d)|| - 1 \right)}
\]

where \(E(S)\) is the number of edges between the nodes in a set \(S\) and \(||S||\) is the number of elements (or cardinality) in that set. Observe that the traditional clustering coefficient is obtained by making \(d = 1\) in the above equation. It can be verified that the hierarchical clustering coefficient of node \(i\) at distance \(d\) quantifies the connectivity between those network nodes which are at distance \(d\) from \(i\). Observe also that \(0 \leq HCC(i, d) \leq 1\), with the null value indicating total absence of connections between the nodes in \(H(i, d)\), while the unit value indicates that each node in \(H(i, d)\) is connected to all other nodes in that set, except itself (loops are not taken into account in this work). Of particular interest is the fact that higher clustering coefficient at a specific distance \(d\) implies the presence of many cycles composed of \(2d + 1\) edges.

Figure 2(c) shows the average value \(\langle HCC \rangle\) (considering each network node) of the hierarchical clustering coefficient for \(d = 1\) to 15. Starting at 0.008 for \(d = 1\), the values of \(\langle HCC \rangle\) then define a plateau extending from \(d = 2\) to 5 and then falls steadily. Such a result provides important insights into the topological organization of the channel system. First, the low clustering coefficient observed for \(d = 1\) expresses the fact that the neighbors of each node are weakly connected one another. This indicates that the channel structure does not involve cycles at such a scale. At the same time, the relatively higher values of hierarchical clustering coefficients observed from \(d = 2\) to 5 implies the existence of several cycles of 5 to 11 edges and provides further indication that the analysed channel system is not a tree.

Generally, the arterial vascularization of the majority of tissues and organs takes place through dichotomic bifurcation, i.e. an artery bifurcates into two arteries, which in turn bifurcate into four arteries, and so on. The obtained experimental results provide cogent indication that the vascularization of the bone structure does not follow such a general rule. A possible explanation for the deviation from the traditional tree and the presence of cycles could be related to different constraints imposed by specific tissues over structural reconstruction after eventual artery obstruction, which can deprive whole regions of proper blood flow. While in tissues other than bones the organism is known to initiate a neovascularization intended to provide bypasses to region irrigation, such schemes are virtually impossible in the short term in bones, which would demand a complex and long reorganization of both Havers and Volkmann channels containing the arteries (recall that the bone matrix is rigid). Therefore, it seems that the particularly intense connections between nodes from the second to the fifth hierarchical levels, as indicated by the obtained hierarchical clustering coefficients, represents a possible redistribution system intended to immediately cope with eventual artery obstruction.

All in all, this work has described how concepts from three-dimensional reconstruction and image analysis, used jointly with state-of-the-art complex network theory, can be effectively applied in order to quantify certain topological features of biological interconnecting systems, particularly the channel distribution network in mammals bones. We have verified that such structures involve the presence of distribution hubs, present a tendency for node connections with degree 2 and 4, and contain cycles with typical lengths ranging from 5 to 11 edges. Important biological implications of such topological features have been identified and discussed. Further related investigations include the possibility to quantify the width and length of each channel in order to infer additional insights about the bone channel system by using weighted and geographic complex networks.

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[1] L.C. Junqueira and J. Carneiro, Basic Histology, McGraw-Hill/Appleton & Lange, New York, (2002).
[2] R. Albert and A.-L. Barabási, Statistical Mechanics of Complex Networks, Rev. Mod. Phys. 74, 47 (2002).
[3] M.E.J. Newman, The Structure and Function of Complex Networks, SIAM Review 45, 167 (2003).
[4] L.A.N. Amarala and J.M. Ottino, Complex Networks: Augmenting the Framework for the Study of Complex Systems, Eur. Phys. J. B, 38, (2004).
[5] S. Maslov and K. Sneppen, Specificity and Stability in
FIG. 1: The three-dimensional reconstruction of the on-fourth of the considered bone channel network (a) and the respectively complex network (b) obtained by projecting the node coordinates along the $z$–axis.

FIG. 2: The dilog plot of the node degree density, shown in (a), suggests a scale-free network model and indicates the presence of distribution hubs. The conditional node degree density is presented in (b), expressing the predominance of connections between nodes with degrees 2 and 4. The average values of the hierarchical clustering coefficients, shown in (c), is characterized by a plateau extending from $d = 2$ to 5.

Topology of Protein, Science 296, 5569, (2002).
[6] L.F. Costa, The Hierarchical Backbone of Complex Networks, Phys. Rev. Lett. 93, 098702 (2004).
[7] L.F. Costa, A Generalized Approach to Complex Networks, cond-mat/0408076
[8] L.F. Costa and R.M. Cesar Jr., Shape Analysis and Classification: Theory and Practice, CRC Press, Boca Raton, FL (2001).
[9] W. Schroeder, K. Martin and B. Lorensen, The Visualization Toolkit, Prentice Hall, Upper Saddle River, NJ (1997).
[10] J.D. Foley, A. van Dam, S.K. Feiner and J.F. Hughes Computer Graphics: Principles and Practice in C Addison-Wesley, Boston, MA (1995)
[11] A.-L. Barabasi, R. Albert and H. Jeong, Mean-Field Theory for Scale-Free Random Networks, Physica A 272, (1999).
[12] D.B. West, Introduction to Graph Theory, Prentice Hall, Upper Saddle River, NJ (2000).