THREE-DIMENSIONAL MICROVASCULAR NETWORKS FRACTAL STRUCTURE: POTENTIAL FOR TISSUE CHARACTERIZATION?

Oana Craciunescu, Shiva K. Das and Mark W. Dewhirst

Department of Radiation Oncology, Duke University Medical Center

ABSTRACT
It has been shown that the fractal dimension of 2D microvascular networks can discriminate between normal vs. tumor tissue (Gazit et al., 1995, 1997). We have determined the fractal characteristics of five 3D microvascular networks and conclude on the correlation between the computed fractal characteristics and the nature of the tissue of origin. The networks considered in the fractal analysis study were one rat tumor network (RT), one nude mouse tumor (NMT), one hamster skeletal muscle (HSM), one rat cremaster (RC), and one rat cerebral cortex (RCC). The networks were digitized in a 3D lattice starting from the known length, diameter and position of each segment in the network. The digitization process was performed such that the ratio between the initial occupation fraction of the vessels in the network and the occupation fraction after digitization is close to one. The resultant cubic lattices were analyzed using the concept of asymptotic fractals. The fractal dimension \( d_f \), and the minimum path dimension \( d_{min} \) (that measures the tortuosity of the vessels) were determined for all the networks. Fractal behavior was noticed on length scales from 1-1.3 decades, dependent on the actual network size. The values obtained for the fractal dimension for the RT, NMT, RCC, RC and HSM microvascular networks are respectively, 2.6, 2.2, 2.29, 2.12 and 2.08. For the minimum dimension the values obtained are: 1.2, 1.1, 1.16, 1.1, and 1.1. By analyzing the available data, preliminary conclusions lead us to believe that a correlation between the fractal characteristics and tissue type might exist. Another important aspect is that the 3D RT microvascular network seems to have a percolation-like scaling which can be beneficial in monitoring the growing pattern using invasion percolation growth models. However, for general conclusions to be drawn, more networks have to be analyzed.

INTRODUCTION
It is accepted beyond any doubt that biological shape is related to function (Gleick, 1987). However, the patterns encountered in biology and medicine are intriguing. In medicine, a decade after Mandelbrot published his physiological speculations some researchers began to find fractal organization controlling structures all through the body. A fractal description fits the data of the bronchial branching, urinary collecting system, biliary duct structure in the liver, blood vessels in the heart, pulmonary blood flow, and placental arterial tree (Bergman and Ulberg, 1998). The structure of dendrites, axon terminals and retinal neurons are fractal objects as well (Alves et al., 1996). Pathologic conditions have also been shown to have fractal characteristics. Certain skin lesions have similarities with computer-generated fractal structures (Keips et al., 1993). Questions have been raised whether a connection between chaos and cancer exists, based on the fractal appearance of tumors that might indicate a power law that governs the tumor growth (Claridge et al., 1992, Sedivy and Mader, 1997, Smolle, 1998). It also has been shown that fractal dimension predicts broadband ultrasound attenuation in stereolithography models of cancerous bone (Langton et al., 1998). Synergistic diagnosis prospectively can be done using the fractal dimension as an imaging parameter in CT scans of patients with anorexia (Hentschel et al., 1998).

Organs, cell tissue, cellular membranes, and the corresponding vascularization form patterns with morphological-functional peculiarities reflecting normal or pathological states (Losa, 1995). The extraction of structural/morphological information relevant for histological diagnosis has been done using fractal analysis. It has been implied in several studies that the fractal dimension (or other fractal characteristics) can be used as a discriminant between different vascular patterns. For example, fractal dimension was found to be a sensitive indicator of optic nerve head neovascularization (Daxter, 1993). Also, local connected fractal dimensions were used to detect and objectively characterize local retinal abnormalities (Daxter and Ettl, 1992). The concept of generalized fractal dimension from the theory of chaos and dynamic systems has been used to discriminate and quantify idiopathic pulmonary fibrosis from normal lungs (Rodriguez et al., 1995). Fractal dimension was also used as a measure of altered trabecular bone in inflammatory arthritis (Caldwell et al., 1998). Tumor vessels are tortuous and sinusoidal, have increased vascular length and diameter compared to normal tissue, and contain shunts, loops and variable intravascular distances (Dewhirst, 1993,
warren, 1979). these characteristics result in biological structures that cannot be easily described using deterministic models. a deterministic model for the tumor vascular network might be possible, but very laborious, because the system to be described is very complex. angiogenesis in tumours encompasses a distribution of patterns with a non-analytical characteristic function. for a deterministic model we need the distribution of the vessels lengths, diameters, and curvatures to determine the flow field. but this might not be enough. it is known that the physiology of the tumor vessel walls is completely different that for normal vessels. in tumors, endothelial cell lining may be incomplete, leading to flow channels through interstitium.

as an alternative, the irregular and fragmented non-euclidean patterns of angiogenesis and perfusion have been described using fractal geometry (mandelbrot, 1982, tsonis and tsonis, 1987, kiani and hudetz, 1991, vico et al., 1992). in particular, percolation theory has been used to describe vascular networks (gazit et al., 1995, 1997, daxter and ettl, 1995, hudetz, 1993, daxter, 1993, hudetz and werin, 1986). baish et al. (1996) used the invasion percolation network model to determine the role of tumor vascular architecture in drug delivery. gazit et al. were the first to show that tumors display "percolation-like scaling" by studying the scale-invariant behavior of normal and tumor vascular networks (1995, 1997). the study used skeletonized images of 2d tumor vascular networks grown in mice bearing dorsal skinfold chambers to observe the tumor vascular geometry. from their observations they were able to conclude that the 2d tumor vasculature could be described using invasion percolation.

in this paper we present preliminary results obtained after applying fractal analysis concepts to 3d microvascular networks with the intent to collect data to support the following two hypotheses:
1. fractal characteristics of 3d microvascular networks can discriminate between normal vs. tumor tissue;
2. tumor microvascular network growth can be described using the invasion percolation growth model (ipgm).

materials and methods
the networks considered in this preliminary fractal analysis study were one rat tumor network (rt), one nude mouse tumor (nmt), one hamster skeletal muscle (hsm), one rat cremaster (rc), and one rat cerebral cortex (rcc).

the rat and mouse tumor networks and the rat cremaster were obtained in the microcirculation laboratory at duke university medical center. details about the network depiction are provided elsewhere (dewhirst et al., 1989, dewhirst et al., 1992, secomb et al., 1993, secomb et al., 1998a, secomb et al., 1998b). briefly, it consisted of the surgical implementation of a transparent window chamber on a dorsal skin flap of a rodent (either nude mouse or fisher 344 rat). for the tumor networks, a 0.1 mm² piece of either ls180 tumor (nude mouse) or a mammary carcinoma (r3230a, fisher rat) was introduced in the window region and allowed to grow for 7-8 days. a region of microvascular network visible in the chamber was observed by intravital microscopy using transillumination of the preparation. real time visualization permitted identification of all vessels in the region of interest.

the three-dimensional vascular network geometry was reconstructed using images registered with a fluorescent confocal microscope, from which images were obtained at successive optical sections (2 μm apart) through the depth of the tissue followed by tracings from the video images and discretization of each two-dimensional slice (secomb et al., 1998a).

the hamster skeletal muscle, and the rat cerebral cortex networks were obtained from the physiology dept. at the university of arizona, tucson. the rcc was deduced from scanning electron micrographs of corrosion casts (motti et al., 1986, secomb et al., 1998b). the three-dimensional arrangements of capillaries in the rsm network was obtained by placing capillaries randomly within the tissue region, subject to certain constraints as detailed in secomb et al., 1994.

the resultant three-dimensional discretized networks were then transformed into a 3d lattice, starting from the known length, diameter and position of each segment in the network. the digitization process was performed such that the ratio between the initial occupation fraction of the vessels in the network, and the occupation fraction after digitization was close to one. due to the fact that not all the vessels were connected, the hoshen-kopelman algorithm is applied to remove any isolated vessels and retain only a connected, percolating cluster (hoshen and kopelman, 1976, binder and heerman, 1992).

initially, the fractal dimension dₙ of each three-dimensional cluster was computed using the sandbox method (bunde and havlin, 1994). however, from the log-log plot of the mass of the cluster vs. the size of the cluster the correlation was not perfectly linear, such that the principles of asymptotic fraxats have to be applied to determine the asymptotic fractal dimension (rigaut, 1984). this approach consists of investigating the first derivative of the log-log data which theoretically approaches a sigmoid curve. the derivatives are determined by calculating the local slopes (through linear regression of three or more consecutive points) along the plot. if the resultant sigmoid has two asymptotes (yₘᵢₓ and yₘᵢₜ), then values of the two horizontal asymptotes correspond to the slopes in the original log-log data (landini and rigaut, 1997). in our case, for the sandbox method: yₘᵢₓ = d, the topological dimension, and yₘᵢₜ = dₜ, the fractal dimension. the final step is to fit the first derivative of the log-log plot to a sigmoid. we applied the logit transform suggested by rigaut (rigaut, 1984, landini and rigaut, 1997) for the so called asymptotic fractal model, i.e.:

$$\logit(y) = \ln \left( \frac{y - y_{\text{max}}}{y_{\text{min}} - y} \right)$$

the fractal dimension of the minimum path, dₘᵢₜ, is computed for each cluster from the power law: lᵢ = rⁿᵈₘᵢₜ. the chemical distance lᵢ is determined using the algorithm proposed by herrmann and stanley, 1988.

results
figure 1a shows the three-dimensional network obtained from the image analysis of the confocal data for the rat tumor (after secomb et al., 1998a, with permission). the digitized version needed for the computation of the fractal dimension is shown in fig. 1b. the final cluster obtained after the hoshen-kopelman algorithm is shown in fig. 1c.
Figure 1  a) Three-dimensional configuration of microvessels in a rat tumor tissue (reproduced with permission from Secomb et al., 1998a); b) Digitized version of the 3D microvasculature in a), obtained from the known length, diameters and position of each segment in the network; c) Final connected cluster obtained after applying the Hoshen-Kopelman algorithm.

For the RT network digitized in Fig. 1c, the log-log plot of the mass of the cluster vs. the size of the cluster (determined with the sandbox method) is shown in Fig. 2a. It can be noticed that the correlation is not perfectly linear (the resultant fractal dimension is \( d_f = 2.01 \)), such that the principles of asymptotic fractals have been applied to determine the asymptotic fractal dimension. By computing the slope of the curve in Fig. 2a, and plot it versus the log of the cluster size, it has been shown that the resultant sigmoid (see Fig. 2b) has two asymptotes: \( y_{\text{min}} \) and \( y_{\text{max}} \). The values of the two asymptotes correspond to the slopes in the original log-log data (Fig. 2a) (Landini and Rigaut, 1997). For the sandbox method, \( y_{\text{min}} = d_t \) (topological dimension), and \( y_{\text{max}} = d_f \) (the fractal dimension), such that, for this specific case, \( d_f = 2.6 \). The minimum path dimension for this example cluster is \( d_{\text{min}} = 1.2 \).

Figure 2  a) Log-log plot (sandbox method) for the network shown in Fig. 1c; b) Local slope of the data in a) (computed using least squares between eight consecutive points) vs. the log of the size of the cluster. The sigmoid shaped curve has two asymptotes, from which \( y_{\text{min}} = d_t = 2.6 \).

Due to the fact that the fractal dimension obtained for the RT network is only within 3% from the fractal dimension reported in literature for 3D percolation (\( d_{\text{ip}} = 2.53 \pm 0.02 \), Jan and Stauffer, 1998), and that \( d_{\text{min}} = 1.2 \) (≠ 2 for diffusion limited aggregation which has the same \( d_f \) as percolation), we applied the invasion percolation growth model (IPGM) to evaluate the growth of the network in time. The method used was proposed by Wilkinson and Barsony, 1984, and a simple example and a description of the algorithm is also shown in Craciunescu et al., 1999. The procedure requires the selection of a starting point (seed of the cluster). For tumor microvasculature, it is hard to determine the feeding point of the tumor. The largest diameter vessel is not always the feeding vessel. In starting the IPGM algorithm for this RT network, we chose as a seed of the cluster the largest diameter vessel entering the cluster from the bottom, as the tumor cells were implanted at the bottom of the window chamber and the growth was presumed to start from there. Figure 3a-f shows a sequence of growing patterns obtained after applying the IPGM to the RT microvascular network. The starting point is marked on the figure, as well as the percentile from the total growing time of each individual growing pattern.

DISCUSSIONS AND CONCLUSIONS

Although this study considered only a limited number of microvasculature beds, certain preliminary conclusions can be drawn in support of the two hypotheses mentioned in the Introduction section.
The RC and the HSM networks (both with the same physiology and in the category of normal tissue) have very similar fractal dimensions (within 2%), and equal minimum path dimensions. This implies that similar tissues could possibly have similar fractal characteristics. In addition, the RT network presented in detail has a fractal dimension and a minimum path dimension closer to 3D percolation, and 20% larger than the two normal muscle microvasculature beds considered in this study. This difference supports our first hypothesis that fractal characteristics can discriminate between normal and tumor tissue vasculature.

As shown here, if 3D tumor microvascular networks have percolation-like scaling, then the IPGM can be applied to describe the growing patterns of these tumor microvascular networks. Such results can help in understanding tumor angiogenesis with multiple applications in the study of tumor physiology. Also, the influence of hyperthermia treatments (cell killing by a sustained high temperature) can be monitored and related to the angiogenesis process, as well as the filling patterns in tumors.

The value obtained for the fractal dimension for the NMT network is sensibly different than that for the RT. Possible reasons for that might be the difference in the tumor lines used, and also the choice of the region of interest containing the network. It is known that tumors tend to be more vascularized in the periphery, hence a network in that region is expected to have a larger fractal dimension.

In addition, the fractal characteristics of different tumor lines may be correlated to physiological properties such as tumor oxygenation and acidity. These two factors are crucial during radiotherapy (cancer treatment using ionizing radiation) and hyperthermia, and not easy to measure during these treatments. For example, Dewhirst et al., 1998 reported that intermittent hypoxia (low oxygen levels) is a common phenomenon in tumors. The authors speculated that a “vascular remodeling” might underlie the mechanism responsible for these large fluctuations, due to the fact that blood vessels are formed and die quickly with implications on the tumor blood flow. Given the association of “vascular remodeling” with the oxygen fluctuations, it becomes reasonably to assume that a correlation between tumor hypoxic fraction and its fractal characteristics exist.

The results presented in this study can be hardly considered as acceptable if not backed up by more similar analyses on different tumor lines and on different examples within the same tumor line. We are currently planning experiments to acquire more 3D tumor networks, and also to register during the experiments the growing patterns for a direct comparison with the results obtained using the invasion percolation growth model.

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