A Fractal Model of Blood Vessel Formation in Porous Polymer Implants

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In this paper the vascularization of typical porous polymer implant used for the dose provision of medicines is presented. Vessel growth was evaluated by simulation of spatial dispersions and nearest neighbor correlations of regional flows in a fractal tree model.

Keywords: polymeric implant, vessel growth, fractal dimension

There have been many exciting advances made in the field of medical implants. Concepts such as biosensors and implantable controlled drug delivery have great promise, but cannot be realized without a clear understanding and control of the biological response. The first implants ever created were bone and joint replacements [1]. Under optimal conditions, there would be minimal scar tissue surrounding these structures. And since these devices were predominantly physico/mechanical in function, the scar tissue never posed a significant problem. This scar tissue and implant-body interaction are respectively called the fibrous capsule and the foreign body response, and were once considered the mark of a biocompatible material. However, this is no longer acceptable for the newer, more sophisticated implant designs. While fibrous encapsulation mattered little with the physical devices, this process disables biosensors and drug delivery devices after a few weeks or months by acting as a barrier which greatly impedes electrical and chemical transmission [2].

Any mechanical response of polymeric structures used and particularly the relaxation produced in time should not be neglected even if both mechanical stress and temperature conditions remain unchanged [3, 4].

As a way of controlling the foreign body response, it may be possible to specially design materials as tissue-implant interfaces. These materials would ideally allow for a permanent, highly vascular tissue to surround the implant. This highly vascular tissue would allow for the rapid exchange of chemical signals, such as drugs and nutrients. To develop this interface, a detailed understanding of both the biology of the tissue response and blood vessel formation is required.

Experimental part

Controlled Drug Release

The goal of controlled drug delivery is to provide a specified drug concentration within the body for an extended period of time. A device that provides a sustained release of drug can maintain desired drug concentrations in the blood with reduced number of doses, while also minimizing the concern of undesirable, sometimes toxic, side effects. Controlled release is, also, a more cost-effective way of delivering expensive medications. However, this is just a subset of the actual goal of controlled release. The primary aim of controlled drug delivery is complete optimization therapeutic delivery; that is the ability to deliver to the desired location, a precise dose for a finite period of time. With this ideal system, one could achieve high bioavailability with minimal side effects and drug exposure. To achieve this idealization, systems must be responsive to fluctuations in the patient's needs. The advantage to implantable drug delivery devices is that they can be designed to meet these aims by providing a means of continually monitored and administered drug delivery.

One type of active system that is currently being developed is the drug array implant [5]. This device is a silicon chip with many tiny reservoirs filled with drug or a microporous membrane where the drug is held. In one system, the reservoirs are coated in a thin nonporous metal layer. When voltage is applied, the metal layer breaks and delivers its reservoir contents. This design holds great promise, as it is capable of rapid on/off delivery. Also, the reservoirs can be filled with many different types of drugs, allowing for complex drug delivery regimes. Determined through several clinical trials, the most common cause of device failure was due to tissue inclusion at the catheter port of delivery caused by the foreign body response [6]. A layer that would allow vascular tissue in growth rather than fibrotic tissue inclusion would be solution to this problem.

For biological systems, chemical communication is the exchange of solutes between cells, tissues, organs, and implanted devices. These solutes can either be nutrients/waste for cellular metabolism or chemical signals that elicit a specific biological response, such as drugs and hormones. To describe the diffusion of a solute to the circulatory system, it is beneficial to divide the process into two parts, diffusion in the bulk tissue and diffusion through the vessel wall [7]. Tissue diffusion is usually modeled as the diffusion of a porous media. The density of the extra-cellular matrix (ECM) proteins, cellular bodies and their orientation regulates the diffusivity. The diffusivity of the tissue decreases as the tissue proteins and cell bodies become more tightly packed. Consequently a loose connective tissue with high vascularity and vessel permeability would provide the fastest route for systemic delivery. It may be possible to

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remodel the tissue surrounding the implant by applying tissue engineering techniques.

The goal of tissue engineering is to repair an existing tissue/organ or completely regenerate a tissue/organ that has failed to function. In order to achieve this, there are two main strategies currently being pursued.

One method is the *in vitro* regeneration of a tissue/organ from primary cells obtained by the patient, and the subsequent reimplantation of the newly generated tissue [8].

The other technique is to implant a device that would temporarily provide or assist the functions of the organ/tissue being replaced, while simultaneously allowing the *in situ* formation of a new organ/tissue [9]. Both of these strategies require a biomaterial scaffold, which organizes the growth of cells into the proper configuration to form the desired tissue [10]. Both of these strategies have been limited by the depth of cellular penetration into the porous networks. It is believed that this limitation is directly related to the depth of penetration of the vascular which penetrates the scaffolding [11]. Without capillaries being fully extended throughout the scaffold, deeper cells will not be able to achieve the required nutrient/waste exchange rates. In order to specifically select vessel growth, an understanding of the physiological pathways of capillary growth is needed.

Blood vessel formation is usually considered to progress through two distinct yet related processes; vasculogenesis and angiogenesis. Angiogenesis is the formation of new blood vessels by the growth of "sprouts" from existing vasculature [12]. This self-limiting process is seen in reproduction, wound repair, and placental development. Vasculogenesis is the developmental formation of vasculature.

Tissue-Implant Interactions

Classic Foreign Body Response

Implants are foreign bodies that will invoke the natural defense mechanism against such intrusions; the inflammatory response. Typically the inflammatory response is split into two categories, acute and chronic inflammation [13]. During the acute phase, an influx of fluid, plasma proteins, and neutrophils enter the wound/implant site. These neutrophils accumulate at the site of implantation and start to phagocytize any small debris/bacteria that are present. After the neutrophils have entered the area and cleared away any debris, granulation tissue (highly vascularized tissue) begins to form, and the natural wound healing response continues. At this point the response can split into either a chronic inflammatory response or a foreign body reaction of the acute type. If there is a constant chemical or physical irritation (as in free movement of the implant), the chronic inflammatory response will occur. If there are no negative chemical or physical signals then classic foreign body response occurs. Typically, the foreign body response results in 3 characteristic layers. A primary layer of macrophages and/or foreign body giant cell formations surrounds the implant. These cells secrete the second layer composed of dense fibrous tissue 30-100 μm in thickness. A third layer of granulation tissue surrounds this fibrous wall. This response is indefinitely stable except for a decrease in cellularity of the primary layer. The dense nature of the fibrous layer greatly impedes the diffusion of most chemical species, as a result prevents any implanted drug delivery device from functioning effectively.

Tissue Response to Porous Materials

The tissue response changes greatly when the implanted material has a porous morphology. It was found that materials with pores bigger 5μ were surrounded by highly vascular loose connective tissue. When the pore sizes further increased, evidence of vascular penetration was evident. It was suggested that the macrophages degree of attachment onto the material surface than dictates the signals that they send out [14]. When the macrophages are able to spread onto the surface of the material, they release signals that call for the deposition of the tight collagen layer. When these macrophages penetrate into a porous sample, and cannot spread fully on the surface, this signal is not released or released to a reduced extent.

Porous Polymer Network Formation

The polymer network was represented as a 3D logical array (100 . 100 . 100) where TRUE represents the presence of polymer, and FALSE no polymer. To generate a porous network, spherical holes (diameter of 1, 3, 5, or 9), were sequentially removed from a solid (all true) polymer array. The location of each hole was randomly selected, and all points described by the sphere were set to zero. This process continued until the desired porosity (50% - 90%) was achieved. Polymer spheres were allowed to overlap to allow for pore size distribution and interconnection. Periodic boundary conditions were used for the formation of the polymer networks. All indices within 2 units from the edge of the polymer were set to true, in order to create "walled" boundary conditions in the random walk simulations. Each network was saved with and without a 3 unit gap between the wall and polymer. This gap was added to represent

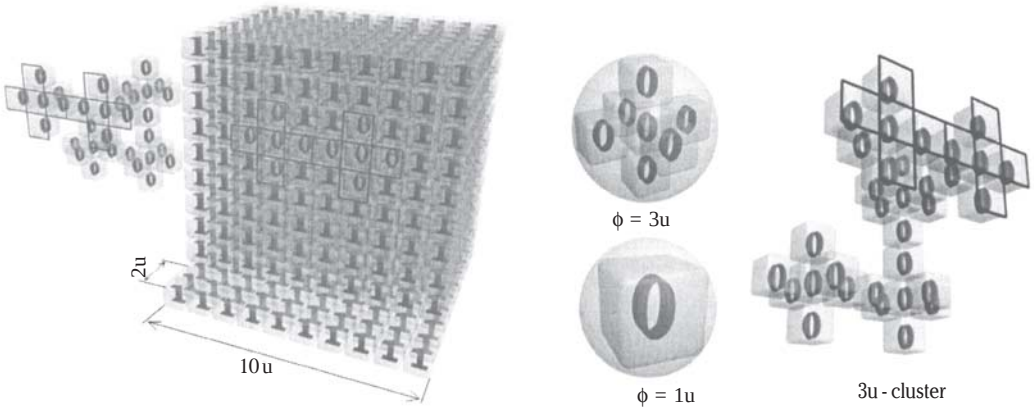

Fig.1 (10. 10 . 10) segment of the main matrix (left) with a porous network of spherical holes (zeros) with a diameter of 3u (right)

external surface available for exterior vascularization. One polymer network was generated for each pore size and porosity.

According to our model, in figure 1 we show a formal representation of the 3D logical matrix. Each cube in the picture stands for 1 bite of memory that will hold the TRUE/FALSE value. For example a spherical pore, with a 3 unit diameter, is a sphere shaped spatial distribution of 7 bits set to zero. A simple array scanning technique was used to obtain statistics on pore size distribution consisting in searching for the 1 (TRUE) values and outputting a sequential index of their locations that is used to find the gap size between each polymer. The 3D structure of the matrix is used to find an orientation bias by applying the algorithm along the three Cartesian axes.

Results and discussion

From *in vitro* and *in vivo* experiments, it is known that pore size and porosity has the greatest effect upon endothelial cell penetration and vascular formation into porous materials. However, the exact mechanism of this relationship is still unknown. It is not fully clear to what extent biological signaling limits growth, or if sieving effects plays any part in endothelial cell penetration [15].

Mathematical models and simulations can be used in conjunction with experiments as a means of evaluating competing mechanistic hypotheses. The analysis of the described polymer shows that the average pore sizes for all polymer networks are larger than the desired pore size. Due to the pore generation technique, no pore sizes smaller than the reference pore is possible so, the networks simulated were considered useful representations of materials with known pore sizes.

Vessel Growth Simulations

The fractal geometry made possible the vascular network quantification by introducing the fractal surface dimension D_s. The computer-assisted analysis showed that, for images belonging to the same blood vessel and under the conditions of same density for all the blood vessels, different values for D_s can thus be obtained. In Figure 2 each blood vessel is represented by a disk. It has been considered that the blood vessels dimension is the same for all the blood vessels. The microscopic images of a vascular system composed of a certain number of blood vessels (between 5 and 30) have been simulated and for each of the generated images the spatial distribution being different. For each of the simulated images the fractal dimension has been evaluated. Due to the fact that the only variable from these images is the blood vessels distribution in space, D_s depends on the irregular arrangement of the blood vessels within the surrounding medium. D_s increases ($p < 0.05$) when images with a greater blood vessels density are being taken into account because the space occupied by the vascular component is bigger; the increased blood vessels density reduces the variation of the space filling properties and the standard deviation.

Figure 3 emphasizes the fractal dimension evolution as function of the vascularization density. As it can be observed by analyzing the graph, under the conditions in which the blood vessels density is the same, for different spatial distributions of them, different values for the fractal dimensions are obtained. Taking into account that the only variable in this situation is the spatial distribution it follows that the fractal dimension depends on the irregular mode of the blood vessels spatial distribution.

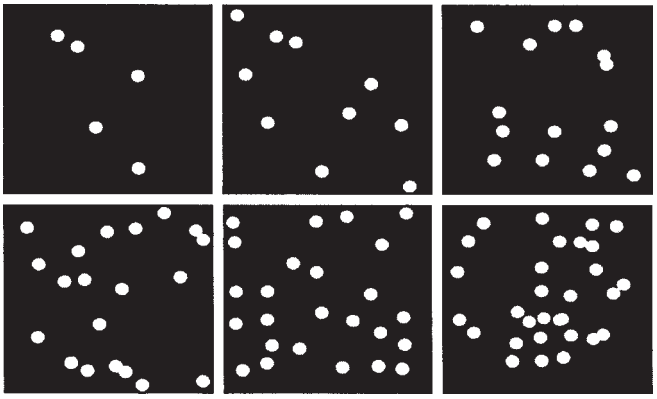

Fig. 2 The modelling procedure of the vascular system's transversal section

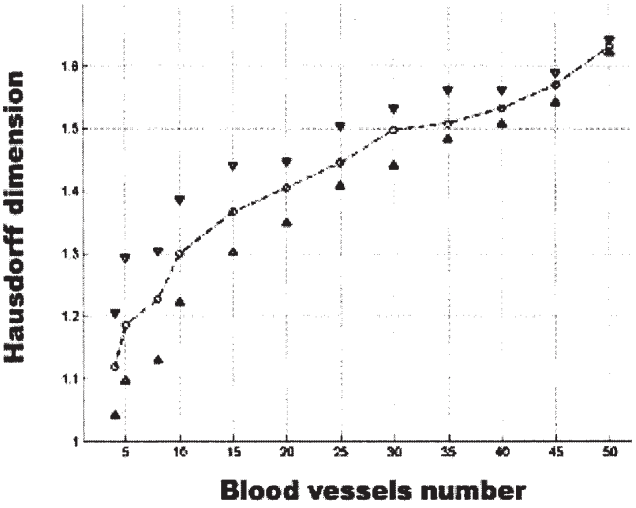

Fig. 3 The fractal dimension behaviour with the increase of the vascularization density

The increase of the blood vessels density reduces the space filling possibility and therefore the standards deviation decreases as well. Due to the fact that the vascular system is a complex network built up of tubes with different sizes, inter-connected and irregularly distributed in the surrounding medium, these geometrical features underline the complexity of the generating process and lead to the conclusion that it would be more efficient for the modelling methods to transform the blood vessels in Euclidean objects. The vascularization can be considered to be a fractal object because it presents an irregular shape, self-similar, real dimension (which doesn't necessarily belong to the integer numbers domain) and depends on the observation scale.

In the present case, D_s will be considered as a spatial complexity measure of the vascularization analyzed in a bidimensional space. The theory that lies at the basis of the D_s definition has been taken from the fractal geometry [16-18]. D_s finds its usability due to the fact that the vascular tree, as any other biological system, can only be analyzed by the measurement of its properties (for example the micro-vascularity density).

Especially D_s is a parameter which depends on:

- the number of blood vessels;
- the spatial relation between the components of the vascular system;
- the interactions between the components of the vascular system and the external environment.

A set of 1000 images that show a different number of blood vessels, randomly distributed within a planar surface have been analyzed and simulated in a more simplified manner, considering that the blood vessels are

tubes with the same diameter, randomly distributed in space and this way it has been observed that D_s increases with the number of blood vessels that form the system. Moreover, the value of this variable modifies when the blood vessels number stays the same. In other words, it is possible that the same number of blood vessels to differently fill up the space as a function of their distribution manner. These results certify the utility of approaching the vascular system from the fractal geometry point of view.

Conclusions

The developed model suggests that D_s can be considered as an estimation of the vascular system geometric complexity, represented in plane. Because the vascular system complexity depends on the number and distribution of the blood vessels, the only use of the micro-vascularization density measure could not emphasize the number of cells that can be accommodated within a blood vessel. On the other hand D_s depends on the continuity and vicinity degree of the blood vessels. These two properties determine the inter-capilar distance and they are not only involved in the vascularization complexity evaluation. The inter-capilar distances are locally defined by both the difference between the pro- and anti-angiogenic factors from each micro-tissue region and by the non-angiogenici factors as well. In a normal tissue, the vascularization density reflects the metabolic rhythm.

Theoretically D_s is higher than 0 (which corresponds to a point in the Euclidean space) and less than 2 (the dimension of a plane). Therefore when it tends to 2, the vascularization geometry tends to fill the bidimensional space and its complexity is higher. The complex geometry of the tumoral vascularization cannot be only measured on the micro-vascularization basis. With the help of the fractal geometry notions, a large scale of morphological structures found in nature, including the vascular networks, can be explored.

The simulation conditions were established in such a way to closely approximate the conditions that are found during typical porous implant vascularization. Since vessel growth is known to occur around and into porous networks, it was hypothesized that by having exterior surface available for vessel growth, the relative amount of vessel growth into and around the polymer block can be calculated. From simulations, site occupancy was not significantly different than that of no polymer network. Moreover, since the system is under confined boundaries this relative occupancy would not be physically meaningful when compared to the unbounded *in vivo* experimental results.

References

1. PARK, J.B., Biomaterials: An Introduction, Plenum Press, New York, 1979
2. SHWARKAWY, A.A., KLITZMAN, B., TRUSKEY, G.A., REICHERT, W.M., J Biomed Mater Res, **37**, 1997, p. 401
3. PAUN, V.-P., Mat. Plast., **40**, nr. 2, 2003, p.81
4. PAUN, V.-P., Mat. Plast., **40**, nr. 1, 2003, p. 25
5. LOW, L., SEETHARAMAN, S., HE, K., MADOU, M.J., Sensors and Actuators B, **67**, 2000, p. 149
6. SAUDEK, C.D., J Clin Endocrinol Metab North Amer, **26**, 1997, p. 599
7. LIEB, W.R., STEIN, W.D., Nature New Biol, **234**, 1971, p. 220
8. WANG, Y., YANG, Z., XIE, H., LI, S., Journal of Biomedical Engineering, **285**, nr. 1-3, 2001, p. 116
9. THOMSON, R.C., MIKOS, A.G., BEAHM, E., LEMON, J.C., SATTERFIELD, W.C., Biomaterials, **20**, nr. 21, 1999, p. 2007
10. FREYMAN, T.M., YANNAS, I.V., GIBSON, L.J., Progress in Materials Science, **46**, nr. 3-4, 2001, p. 273
11. GRIFFITH, L.G., NAUGHTON, G., Science, **295**, 2002, p. 1009
12. BERTOLINI, F., MANCUSO, P., GOBBI, A., PRUNERI, G., Experimental Hematology, **28**, 2000, p. 993
13. ANDERSON, J.M., Cardiovasc Pathol, **2**, nr. 3, 1993, p. 33
14. PADERA, R.F., COLTON, C.K., Biomaterials, **17,** 1996, p.277
15. SIEMINSKI, A.L., GOOCH, K.J., Biomaterials, **21**, 2000, p. 2233
16. OLTEANU, M., PÃUN, V.-P., TANASE, M., Rev. Chim. (Bucuresti), **56**, nr. 1, 2005, p. 97
17. IORDACHE, D., PUSCA, S., TOMA, G., PÃUN, V.-P, STERIAN, A., MORARESCU, C., Lect Notes Comput Sc 3980, 2006, p. 804
18. PÃUN, V.-P., OLTEANU, M., MARIN, A., Rev. Chim. (Bucuresti), **58**, nr. 3, 2007, p. 355

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