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On the growth model of the capillaries in the porous silk fibroin films

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Abstract This paper discussed the random growth model of the capillaries in its growing process, analyzed the relations between the growth of the capillaries, the metabolism of the organism tissue, and micro environmental condition and secretion of the growth factors. Furthermore, the paper discussed the growth law of the capillaries in the porous silk fibroin films (PSFF) in order to provide a theoretical basis for the designing and making of the new biomaterial of the PSFF more suitable for the growth of the cells and capillaries.

In order to work out a biomaterial which will be very compatible with the organism tissue and helpful to its growth and repair, it will be a necessity to find out the microcosmic behaviors of body fluid, cells, and capillaries in the contacting interface between the biomaterials and the tissue. Using the porous protecting film made of silk fibroin to protect the wounded area in the animal experiments, we find that it has good biology security, and that the capillaries and the fibroblasts can grow into the pores of the silk fibroin film [1], but the growth of the cells and the capillaries have obvious selectivity to the interstice rate and the pore size of the PSFF [1]. That's to say, cells can grow well into some PSFFs with some certain porous structures, and cannot grow into films with other porous structures and even that tissue necrosis may occur. Till now the cellular biology mechanism of this phenomenon is still not elucidated. In order to design and make a more rational PSFF with its porous structure more suitable to the growth of the cells and tissue, especially in order to obtain different PSFF materials suitable to the repair needs of different tissue and parts, it's important that we should study and discuss the microcosmic growing behaviors of the tissue and capillaries in the PSFF structure, find out and establish the growth mode of the cells and capillaries in the biomaterials. The research hereby discussed the growing of the cells and especially the angiogenesis in the PSFF, focused on the growth model of the capillaries during their formation, tried to elucidate the relation between the growth of the capillaries and the structure of the biomaterials in the PSFFs.

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Growth model of the capillaries in the PSFFs

Firstly make a model to describe the growing process of the cells and the capillaries in the PSFF materials that are implanted into the tissue. The angiogenesis in the PSFF discussed here is a process that on the basis of the existed capillary network, the endothelial cells expand, transfer and unite one another to form a new capillary vessel. With the help of the present relevant theories about the growth of the capillaries, this process can be disassembled into the following steps:

(1) After the implantation of the PSFF materials into the internal tissue, the organism tissue fluid infiltrates into the pores of the films and forms an environment suitable for survive of the cells.

(2) When the pore size of the PSFF is big enough, cells and some cellular genes will infiltrate into the pores with the tissue fluid.
(3) With the broaden of the spreading area of the tissue fluid and cells, the distance of the material exchange of the cellular breath and metabolism between the tissue fluid and capillary bloods will increase, will make the diffusing distance of oxygen, nourishment and cellular metabolite go beyond the limit, and thus the metabolic efficiency will decline, the cells will be in a hypoxia state.
(4) When the cells in the PSFF go into a hypoxia state, the transcription factor and hypoxia inducible factor-1 (HIF-1) [2] will be activated, which will speed the expression of the growth factors of the blood vessels such as vascular endothelial growth factor (VEGF) and Angiopoietin-2 (ANG-2)[3, 4]. At this time the cells will compose and release growth factors of blood vessels, such as VEGF, ANG-2 [5].
(5) Under the action of the VEGF, the periphery cells of the nearest capillaries in the organ will separate; the endothelial cells proliferate and move to form a reticulation configuration in the shape of cavity, and eventually connect and expand into random capillary network [6–8].

In the human body, the circulation distribution of the capillaries presents a very complex state, and there is not a fixed circulation mode, different tissue organs have their specific microcirculation structure modes that are related to the structures and functions of all kinds of tissue. But the growth of capillaries namely the formation of the network still have some common characteristics. For instance,

(1) With bounds, capillaries in certain tissue can only grow in certain tissue and within the boundaries of the organs.
(2) With limitation, VEGF is a molecule signal of the cellular paracrine of the blood lack tissue [9]; VEGF only can diffuse in a certain area and lingers nearby the cells that secrete these signal molecules, the limited diffusing distance is about 100–200 μm, which is the reason that only the implantation of thin artificial skins is easy to repair the wounded surface.
(3) Within a degree, when the capillaries are dense enough to solve the problems of blood lack and oxygen lack of the cells, the producing and secretion of the genes that promote the growth of the capillaries will be restrained.
(4) Unification between order and disorder, the capillary networks in the organs with different structures have their certain configurations, the different configurations all take on a random growing state and at the same time abide by the rule of material saving (such as the the vascular endothelial cells), and fulfill the aim of efficacy.

Based on the above growth modes and characteristics, firstly we will discuss the growth density of the capillaries, which is a factor that has direct relation with the needed porous state of the PSFF.

Analytical model of the growth density of the capillaries

The growth of the capillaries in the wound repairing tissue or the PSFFs has direct relation with the oxygen consumption state of the cells in the tissue fluid. Therefore, the density of the capillaries in different organ tissue has great difference due to different speed of metabolism. For instance, if we use the maximum number of the capillaries in the transect of every cubic millimetre tissue to express the density of the capillaries, there will be 2,000–3,000 capillaries in some parts of the human body with high capillary density; while in the dermis and connective tissue there are only about 50 capillaries [10]. In order to analyse the relations between the density of the capillaries, oxygen supply of the capillaries and oxygen consumption state of the tissue cells, we consider a simplified oxygen supply model of the capillaries in the mode of diffusion. Figure 1 is the sketch map of a capillary vessel and its periphery tissue system. The capillary's radius is r_0, and its length is l.

We make a basic supposition that when the oxygen supply from the capillary and the oxygen consumption of the tissue come into a steady state, the oxygen concentration in the capillary is a fixed value u_0, while the diffusing intensity of the oxygen v_0 in the capillary wall is in proportion to the oxygen consuming intensity g in the periphery tissue around the capillary. At the moment in the annular area of the tissue with a radius of r and a thickness of Δr, the entering oxygen quantity equals the consuming oxygen quantity of the tissue cells plus the diffusing oxygen quantity out of the annular area. Namely, if we let the intensity of the oxygen entering into the area through the column side be $v\ (r)$, while the intensity of the oxygen diffusing outside the annular area through the column side be $v\ (r + \Delta r)$, then we have

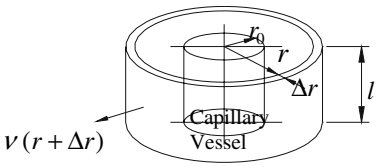

Fig. 1 Oxygen supply model of the capillary

$$2\pi rlv(r) = 2\pi r\Delta rlg + 2\pi(r+\Delta r)lv(r+\Delta r)$$

When $\Delta r \to 0$, hereby we can get the following differential equation

$$\frac{dv}{dr} = -\frac{v(r)}{r} - g \tag{1}$$

Solving the equation, we can get the diffusing intensity of the oxygen in the column side with a radius of r

$$v(r) = \frac{r_0}{r}v_0 - \frac{gr^2}{2}\left(1 - \frac{r_0^2}{r^2}\right); \tag{2}$$

If we let the oxygen concentration in radius r be $u(r)$, then by diffusion law

$$v(r) = -\rho\frac{\partial u(r)}{\partial r},$$

We get

$$u(r) = u_0 - \frac{r_0(2v_0 + gr_0)}{2\rho}\lg\frac{r}{r_0} + \frac{g}{4\rho}(r^2 - r_0^2). \tag{3}$$

In the equation, ρ is diffusion coefficient; u_0 is the oxygen concentration in the capillary. The Eq. (3) is just a special example of the Krogh's Model [11]. If the oxygen concentration in radius r is $\beta\, u_0$, here β is the decline rate of the oxygen concentration, and then we can find the radius $r(\beta)$ at the moment by numerical calculation. When β equals the lower limit of the decline rate β_0 (considering the together effect of the oxygen supply from the adjacent capillaries), we can find the maximum density of the capillaries in an area unit of the transverse section.

$$\varphi = \frac{1}{\pi r^2(\beta_0)} \tag{4}$$

This is a quantity that has relations with the oxygen supply concentration u_0 of the capillaries and the oxygen consumption rate $g$of the tissue.

The construction of capillaries in the PSFF

When tissue fluid, cells infilter into the PSFF or other biomaterials that are implanted into the organism, with the depth of the entrance becoming deeper, the oxygen supply to the cells and tissue will decrease. In the area of slight hypoxia, the compensation reaction is generated in the organism to cause the growth of the capillaries; while in the serious hypoxia area the function and metabolism of the cells will go wrong, and the cellular tissue and structure will be damaged and necrosis. Between the two areas is a region where the cells and tissues in the PSFFs can survive. In such area, the mechanism of the angiogenesis and that of the general angiogenesis are the same. Now we have known that in the angiogenesis of the capillaries, because of the inducement of VEGF, the endothelial cells present a series of special and complex deeds, including proliferation, transfer, co-adherence among the cells, the deeds that the cells stand in lines and form an open cavity structure [6, 12]. However, under the regulation of the internal gene and the action of exterior regulating factor, how the capillaries grow into a three-dimension capillary network with a certain shape and a certain density is still under elucidation [13]. Hereon based on the fore mentioned basic characteristics of the growth of the capillaries, we try to elucidate the mechanism and mode of the network formation of the capillaries in the course of the angiogenesis and the repairing of the wound. The mode we established mainly including the following hypotheses and rules.

(1) In the area with enough oxygen, the endothelial cells become conglobation, have the probability to accumulate [14], and tend to move to the area with meager oxygen [14, 15].
(2) In the area with meager oxygen the endothelial cells try to spread to enhance the ability of absorbing oxygen, and do not grow to overlap each other.
(3) In the serious hypoxia area, soon the endothelial cells will become necrosis due to hypoxia.
(4) The endothelial cells that move to the hypoxia area will assemble in line on the border of slight hypoxia area and serious hypoxia area, and subsequently join each other, and curl into a hollow lumen shape.
(5) The formation of the capillary network is a process that forms layer by layer from hypoxia area to external layer, and the three-dimension capillary structure is formed layer by layer.

These rules ensure that in the time of the wound repair and angiogenesis no new capillaries will produce in the tissue full of oxygen, which make the formation of the capillary network accord with the rule of material saving, and thus the endothelial cells are used in high efficiency. Moreover, these rules make the density of the new capillaries accord with that of the peripheral capillary or match with the state of oxygen consumption and supply (such as in the tumor tissue). On the other hand the capillary network formed according to the above-mentioned rules also takes on a random distribution, because of the dynamic change of the oxygen consumption state in the tissue. Many facts may prove these rules. For instance, Stratmann and other persons put the endothelial cells and the gelatinous tumor cells together into matrigel, and found that endothelial cells transferred and formed capillary network that

anastomosed each other, and the capillaries appeared like a cord; whereas when the gelatinous tumor cells were removed, the endothelial cells would accumulate to agglomerate, the capillaries would stretch abnormally [14]. In the above-mentioned rules, some facts are that we are seeking further confirmation, among which the mechanism that the endothelial cells tend to move to the hypoxia area is a subject that needs study.

According to the above-mentioned constructing rules of the capillaries, we can find the growing state of the capillaries in the PSFFs. Its main characteristics are that, the growing density of the capillaries in the PSFF tends to be the same as that of the implanted tissue; the capillaries take on a random growing state abided by the material saving rule; when the cell in the PSFF is in the serious hypoxia area and its time limit of enduring hypoxia is less than the time of the angiogenesis, the cells will not survive. When the PSFF is implanted in the tissue under dermis, there's still time for the capillaries to grow one to two layers. Thereby we can know that as a PSFF that will be implanted into the organism, its pore size should first afford space for the mesenchymal cells and the protein fiber cells of the nearby tissue to enter, thus causes the increasing of VEGF, and starts the growing of the capillaries. Furthermore the size and depth of the pores should be suitable for the cells to breath under certain oxygen concentration, if the depth of the pores is too deep to afford enough oxygen and nutrition for the growing of the capillaries, there will be necrotic cell layer in the internal tissue in stead of the formation of healthy tissue. According to reference [1], when we implant PSFF with a certain pore diameter beneath the dermis, if the diameter of the pores is 80 μm, the growing of the tissue cells and the capillaries will be bad; if the diameter of the pores is 100 μm or 175 μm, the growing of the tissue cells and the capillaries will be all right.

Discussion and review

Based on the above-mentioned oxygen diffusion mode of capillaries, here we made some simulating quantitative computations about the diffusing state of oxygen in the tissue to find the effects of all kinds of factors to the growth of the capillaries.

The relation between the oxygen diffusing intensity and the diffusing radius

Figure 2 exhibits the curves that the oxygen diffusing intensity falls with the diffusing radius. In the computation we suppose the radius of the capillaries is 3 μm. The curves in the figure are the results when the intensity of the oxygen consumption g is 0.2, 0.3, and 0.5 units. Because the intensity of the oxygen diffusion v_0 is in proportion to g, we can see that when the oxygen consumption intensity of the tissue is high, the oxygen diffusing intensity will also be high, thus the need of oxygen can be satisfied. And the higher the oxygen consumption intensity is, the higher the falling velocity of the diffusing intensity beside the capillaries will be, and the diffusing intensity far away from the capillaries will also be low and even.

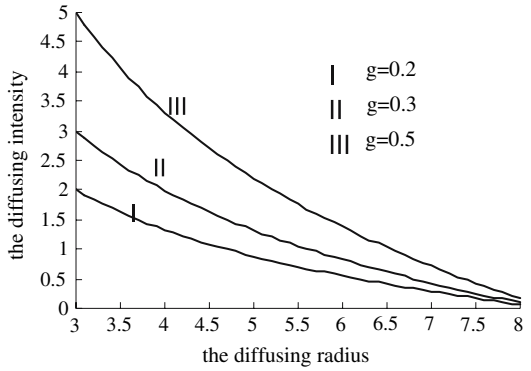

Fig. 2 The relation between the oxygen diffusing intensity and the diffusing radius

The relation between oxygen concentration and the diffusing radius

Figure 3 exhibits the curves that the oxygen concentration falls with the diffusing radius. All the conditions are the same as these of Fig. 2, and the curves show the respective results when the oxygen concentration in the capillaries is 12 units, and the intensity of the oxygen consumption g is 0.2, 0.3 and 0.5 units. From the figure we can see that the oxygen concentration decrease quickly with the increase of the oxygen consumption of the tissue, and it's very obvious in the same radius. It's noticed that when the muscle tissue changes from a rest state into a moving state, the working rate of ATP may increases several times, and even hundreds of times [16, 17], at this moment the oxygen concentration in the tissue will decrease greatly and that the tissue will be in a hypoxia state, and soon it will cause compensation reaction. Thus in the PSFF keeping the tissue in a rest state is useful to the growth of the tissue cells.

The relation between the density of the capillaries and the intensity of the oxygen consumption

In the condition that the oxygen concentration in the capillaries is steady, the range of slight hypoxia and its corresponding density of the capillaries will change with the intensity of the oxygen consumption. From Eq. (3, 4) we can get the diffusing radius r (β) and the density of the

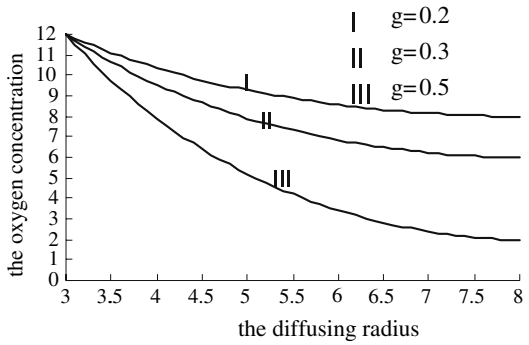

Fig. 3 The relation between oxygen concentration and the diffusing radius

capillaries when the falling rate of the oxygen concentration is β. Figure 4 (a) exhibits the relation curve between the slight hypoxia radius $r(\beta)$ and the intensity of the oxygen consumption when the hypoxia rate reaches $\beta = 0.6$. Here the slight hypoxia radius $r(\beta)$ gives an annular area, in the circumference of which the slight hypoxia rate is β. Figure 4 (b) exhibits the corresponding density curve of the capillaries. Here the abscissa is the intensity of the oxygen consumption g, the ordinates are the diffusing radius and the density of the capillaries respectively. The figures show the results respectively when the initial oxygen concentration u_0 is 30, 36 and 42 units. From the figures we can see that with the increase of

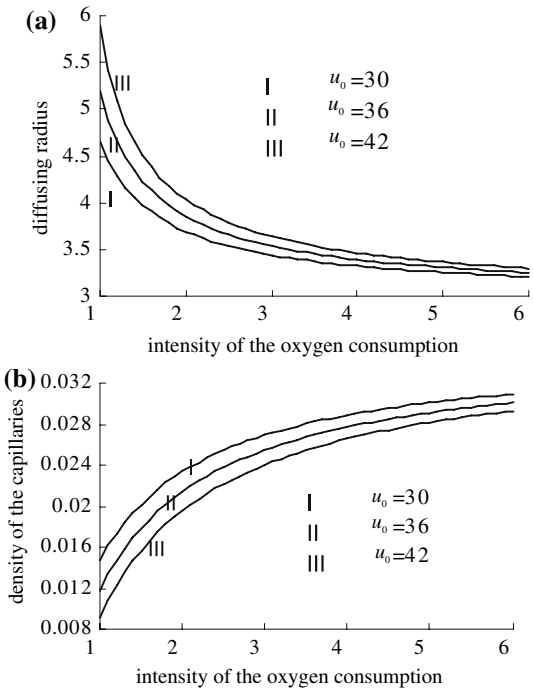

Fig. 4 (**a**) The relation between the slight hypoxia radius and the intensity of the oxygen consumption; (**b**) The relation between density of the capillaries and the intensity of the oxygen consumption

g, the slight hypoxia radius falls quickly, and its corresponding density of the capillaries increases quickly. When g increases to a certain extent, the capillary density tends to be stable. At the same time we can see that the capillary density falls with the increase of the initial oxygen concentration u_0. It is caused by sufficient supply of oxygen in a time unit due to the high pressure of the oxygen supply.

Conclusion

By Modeling and analyzing the growing state of the capillaries in the PSFF, the paper brings forward a spatial structure model of the capillaries, and points out that this is a spatial network that accords with material saving rule and at the same time satisfies the needs of oxygen supply; and elucidates the relation between the mode of the oxygen diffusing around the capillaries and all factors that affect the density of the capillaries.

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