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Dose response of angiogenesis to basic fibroblast growth factor in rat corneal pocket assay: II. Numerical simulations

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Abstract

Angiogenesis involves interactions among various molecules and cells. To understand the complexity of interactions, we developed a mathematical model to numerically simulate angiogenesis induced by basic fibroblast growth factor (bFGF) in the corneal pocket assay. The model considered interstitial transport of bFGF, cellular uptake of bFGF, and dynamics of vessel growth. The model was validated by comparing simulated vascular networks, induced by bFGF at three different doses: 5 ng, 15 ng, and 50 ng, with experimental data obtained in the first part of the study, in terms of migration distance of vascular network, total vessel length, and number of vessels. The model was also used to simulate growth dynamics of vascular networks as well as spatial and temporal distribution of bFGF, which could not be measured experimentally. Taken together, results of the study suggested that the coupling between diffusion and cellular uptake of bFGF was critical for determining structures of vascular networks and that the mathematical model was appropriate for simulation of angiogenesis in the cornea.

Keywords: Mathematical modeling; Diffusion; Vascular network; Vascular density

Introduction

Angiogenesis involves interactions among various molecules and cells, including angiogenic promoters/inhibitors, extracellular matrix, endothelial cells, pericytes, and smooth muscle cells (Carmeliet and Jain, 2000; Folkman, 1997; Hanahan, 1997; Jain, 2003; Neufeld and Kessler, 2006; Risau, 1997). The interactions are intricately coordinated at both molecular and tissue levels, which are critical for the control of growth and maturation of new vessels. The extent of interactions depends on individual components and their distributions, such as concentration gradient of angiogenic factors (Mac Gabhann et al., 2006; Tong and Yuan, 2001). Variations in the interactions can result in completely different vascular networks, including abnormal ones in diseases (Carmeliet, 2003; Neufeld and Kessler, 2006). Due to the complexity of interactions, the final vasculature can hardly be predicted from experiments designed for investigating isolated components in angiogenesis. In this regard, mathematical modeling, which can integrate multiple molecular and cellular mechanisms into a comprehensive system, is an alternative or may be the best approach to understanding angiogenesis as a whole.

Over the past 30 years, a number of mathematical models have been developed for analyzing angiogenesis under various physiological and pathological conditions, such as wound healing, embryonic development, and tumor growth (Balding and McElwain, 1985; Chaplain, 1995, 2000; Chaplain et al., 2006; Liotta et al., 1977; Mantzaris et al., 2004; Stokes and Lauffenburger, 1991). In spite of their success in analyzing angiogenesis, most models are focused on individual mechanisms of angiogenesis but few of them have included necessary details required to directly simulate vascular networks observed in experiments (Peirce et al., 2004; Sun et al., 2005). For instance, angiogenesis in two-dimensional models is commonly assumed to be induced from a straight vessel in a rectangular region. In addition, concentration distribution of angiogenic factors is often de-coupled from interactions between these molecules and endothelial cells rather than evolves with

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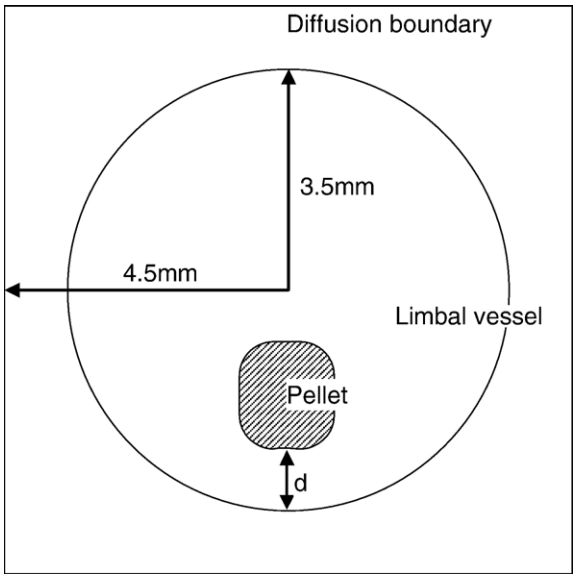

Fig. 1. Model geometry. The cornea was assumed to be a circular disk surrounded by the limbal vessels. The open rectangle denotes the diffusion region for bFGF. The shaded disk represents the pellet.

angiogenesis. To overcome these limitations, a mathematical model was developed in a previous study to simulate angiogenesis in the corneal pocket assay (Tong and Yuan, 2001). The model was built with the geometry similar to that in experiments and considered the coupling between transport of angiogenesis factors and growth of vascular networks. As a result, it was able to predict growth of vascular networks induced by basic fibroblast growth factor (bFGF) in the corneal pocket assay. The goal of the current study was to experimentally validate the model by investigating dose response of angiogenesis to bFGF. It was divided into two parts. In the first part, we quantitatively measured the induced vascular networks in the cornea (Tong and Yuan, 2008). In the second part of the study (i.e., this paper), we modified the previous model in (Tong and Yuan, 2001) by including additional details involved in angiogenesis: (a) an explicit consideration of bFGF transport in the pellet that was coupled with transport of bFGF in the corneal stroma; (b) a new model of bFGF uptake by endothelial cells; and (c) a new model of dose response of endothelial cells to bFGF treatment. The new model was used to numerically simulate the dose response of angiogenesis to bFGF. It was observed that the simulated results were quantitatively in good agreement with the experimental data, in terms of migration distance of vascular network, total vessel length, and number of vessels. In addition, the model was used to simulate growth dynamics of vascular networks as well as spatial and temporal distribution of bFGF, which could not be measured experimentally.

Materials and methods

Development of mathematical model

Angiogenesis in the corneal pocket assay can be induced by angiogenic factors released from tumor cells or a polymeric pellet implanted in the corneal stroma (Polverini et al., 1991). To mathematically model the angiogenesis, we considered (i) interstitial transport of bFGF, (ii) cellular uptake of bFGF, (iii) probability of sprout formation, and (iv) rate and direction of vessel growth in response to bFGF stimulation. The mathematical model was developed by modifying a previous model published in (Tong and Yuan, 2001). To simplify the model description, only the modified parts are discussed in detail and the unchanged parts of the model are just mentioned here.

Model geometry

Angiogenesis and bFGF transport in the cornea were assumed to be two-dimensional (Fig. 1). The model geometry was based on experimental observations in the *in vivo* study (Tong and Yuan, 2008). The radius of the cornea was 3.5 mm. The width and the area of the pellet were 1.5 mm and 2.5 mm^2, respectively. The vertical distance between the pellet and the limbus, d, in each dose group is listed in Table 1 since it varied in different experimental groups in the *in vivo* study.

Distribution of bFGF in the cornea

Distribution of bFGF in the cornea depended on release of bFGF from the pellet, diffusion of bFGF in the cornea, inactivation of bFGF, and uptake of bFGF by vascular endothelial cells. It was assumed that the inactivation rate was proportional to local concentration of bFGF (Tong and Yuan, 2001). Therefore, the mass balance equation for bFGF in the cornea was derived as

$$\phi_c \frac{\partial C}{\partial t} = \phi_c \cdot D_c \cdot \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - k \cdot \phi_c \cdot C - u_v \quad (1)$$

where C is the interstitial concentration of bFGF; ϕ_c and D_c are the available volume fraction and effective diffusion coefficient of bFGF in the corneal stroma, respectively; k is the rate constant for inactivation; and u_v is the rate of bFGF uptake by vascular endothelial cells.

The uptake of bFGF is mediated by two types of molecules on the plasma membrane of endothelial cells: bFGF receptors (FGFR) and heparan sulfate proteoglycans (HSPG) (Bikfalvi et al., 1989; Fannon and Nugent, 1996; Gleizes et al., 1995; Moscatelli, 1988; Murono et al., 1993; Nugent and Edelman, 1992; Reiland and Rapraeger, 1993; Roghani and Moscatelli, 1992; Rusnati et al., 1993). The uptake is triggered by binding of bFGF to these molecules, which leads to rapid internalization of the complexes. After internalization, the complexes dissociate in endosomes and a portion of free bFGF molecules are transferred into the nucleus and the rest is transferred into lysosomes for degradation (Bikfalvi et al., 1989). As a result, the internalized bFGF is irreversibly removed from the extracellular space (Moscatelli, 1988).

By using a wild type Chinese hamster ovary cell (CHO) and a CHO cell transfected with FGFR, Roghani and Moscatelli have observed that the rate of bFGF internalization via the HSPG pathway is linearly proportional to extracellular concentration of bFGF in a range from 0 to 50 ng/ml, whereas the rate of internalization via the FGFR pathway increases nonlinearly with bFGF concentration and reaches a plateau at 5 ng/ml (Roghani and Moscatelli, 1992). This is because there are 10^5–10^6 copies of HSPG per cell (Yanagishita and Hascall, 1992), but less than 2×10^4 copies of FGFR per cell even on the

Table 1
Group dependent model constants[a]

Constant	5 ng	15 ng	50 ng
Distance between the pellet and the limbus, d	0.9 mm	1.06 mm	1.2 mm
Vessel diameter, d_v	12.3 μm	16.9 μm	17.9 μm
Initial concentration of free bFGF in pellet, C_0	58.9 ng/ml	177 ng/ml	589 ng/ml

[a] There were three simulation groups, in which the doses of bFGF were 5 ng, 15 ng, and 50 ng, respectively. The data of d and d_v were measured experimentally in (Tong and Yuan, 2007).