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HOPF POINT ANALYSIS FOR ANGIOGENESIS MODELS

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Abstract. In this paper we present several ODE systems encoding the most essential observations and assumptions about the complex hierarchical interactive processes of tumor neo-vascularization (*angiogenesis*). From experimental results we infer that a significant marker of tumor aggressiveness is the oscillatory behavior of tumor size, which is driven by its vascularization dynamics. To study the forces underlying these oscillations we perform a Hopf point analysis of the angiogenesis models. In the analyzed models Hopf points appear if and only if a nontrivial set of time-delays is introduced into the tumor proliferation or the neo-vascularization process. We suggest to examine in laboratory experiments how to employ these results for containing cancer growth.

1. **Introduction.** Growth of malignant tumors beyond the diameter of $1-2mm$ critically depends on their neo-vascularization, which provides vital nutrients and growth factors, and also clears toxic waste products of cellular metabolism [12]. Indeed, the role of angiogenesis - the formation of new blood vessels by budding from existing ones - as a target for cancer therapy, is currently a focus of intensive research [12],[8],[19].

In order to establish successful anti-angiogenic treatment rationale, the dynamics of angiogenesis must be better understood. These dynamics are very complex, involving many interacting oscillatory processes, which operate on several scales of time and space. Their essential constituents are briefly described below.

Having reached a certain size and, therefore, a certain critical volume/surface ratio, a shortage of oxygen (denoted *hypoxia*) and nutrients is created within the tumor. Under hypoxia the tumor produces proteins, notably Vascular Endothelial Growth Factor ($VEGF$). Increasing $VEGF$ levels lead to increased proliferation and mobility of endothelial cells, and, as a result, to increased formation of immature vessels by these cells. Consequently, the blood supply of the tumor is augmented, encouraging tumor proliferation [22],[11],[25],[23]. The protein $VEGF$ also ensures integrity of immature vessels, and its insufficiency leads to their regression [5],[18],[17]. Immature vessels undergo a process of maturation by attaching

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a layer of pericytes. Vessel maturation is enhanced by the binding of the protein Angiopoietin-1 ($Ang1$), which is secreted by tumor cells, to $Tie2$ receptors on endothelial cells [10],[24]. Both the immature vessels and the tumor produce the protein Angiopoietin-2 ($Ang2$), which blocks the $Ang1 - Tie2$ bond, thus stopping the maturation process and inducing the reverse process - the destabilization of the mature vessels [21],[20],[1]. It appears, then, that $Ang1/Ang2$ ratio determines which of the processes - maturation or destabilization is the dominating one [2].

For enabling the analysis of the complex interactive set of hierarchical processes involved in angiogenesis, the above description has been converted into a detailed discrete mathematical model [2]. Computer simulations of the model suggest that, under certain conditions, tumor size is not a monotonic function of time, but, rather, has an oscillatory nature. In particular, the simulations suggest that tumor growth is maximized under medium levels of $Ang1$ production, and minimized under both high and low $Ang1$ levels [3]. This result is supported by experimental data on over- and under-expression of $Ang1$ in animal cancer models [16]. Its significant implication is that potential anti-angiogenic drugs, which influence $Ang1$ production, can have both stimulatory and inhibitory effects on tumor growth.

The above model manifests very rich dynamics, and it may be advantageous to know which of these dynamics mirror real-life phenomena, crucially affecting the tumor pathology, and which apparent dynamics are merely arbitrary model's artifacts. It seems, however, that the discrete model is much too complex to be tractable to such analysis. Therefore, in the present work we set upon ourselves the task of investigating a much simpler family of angiogenesis models which still captures the essential properties of the system.

What are the essential properties of angiogenesis? This problem was studied by analyzing experiments, where magnetic resonance imaging (MRI) was used for measuring tumor growth and blood vessel density in human epithelial ovarian carcinoma spheroid xenografts [13]. In the analysis of these experiments tumor size was measured as a function of various vessel density parameters evaluated at different preceding time points. Results show that when tumor size was correlated with vessel measurements with a delay of 3 days, both tumor size and vessel density showed oscillations with a relatively fixed amplitude. This observed oscillatory behavior suggests an interdependent path of development for these two processes (Merbl, Arakelyan, Agur, in preparation). Moreover, the analysis of the empirical results suggested that tumor growth-rate decreased upon increasing amplitude and frequency in vessel density fluctuations and upon decreasing compliance between the mature and immature vessel density oscillations. From these results we infer that the oscillatory pattern of vascular density has a significant effect on tumor aggressiveness [4].

These results imply that the oscillating state of the tumor is more desirable, from the medical point of view, than the monotonically growing one, presumably because oscillations prolong the nonterminal phase of the disease. We can ask what are the conditions and feedback loops which make oscillations possible and whether it is possible to preserve the tumor in such an oscillating state by therapeutic means for an indefinite time. This leads us to define simple angiogenesis models in terms of time-delay ordinary differential equation systems.

In this paper we introduce multi-parameter families time-delay ODE systems, which encode the basic known facts and assumptions concerning tumor induced angiogenesis processes. We believe that for these models it makes most sense to ask qualitative questions involving behavior of the system locally with respect to

time. Examples of such questions are existence and stability of fixed points and existence of Hopf points. Being interested in periodic or quasi-periodic behavior of the tumor we investigate the models with respect to existence of Hopf points (see Section 3 for description). Our goal is to determine whether Hopf points exist for the given models. Indeed, biologically relevant fixed points (with positive tumor size) are Hopf points if and only if there is a nonzero time-delay.

2. **General assumptions of the models.** Each of the models herein described involves the following time dependent variables:

- the number of tumor cells or tumor size (denoted by N),
- the concentrations of growth factors known to be involved in tumor angiogenesis, defined as P. For a more accurate description, P may be broken down into several growth factors (proteins) which may differ in effects and/or kinetics,
- the volume of blood vessels feeding the tumor which, again, may either be defined separately as the volumes of immature and mature vessels, or as the total of both, denoted by V.

All modelling alternatives are systems of ordinary differential equations with or without time-delay. In all of these models we use "sigmoid like" functions - smooth monotonous functions having a horizontal asymptote, e.g. $\frac{1}{1+e^{k(x+s)}}$. These functions describe a response of the system to the relevant biological stimuli. The reason for such a choice of the response function is the experimental observation that, below and above certain thresholds, changes in the intensity of the stimuli have minor effects on the response. Between the threshold values (in the sensitivity region), the process rate monotonously depends on the stimuli value. In our analysis we use only the basic properties of sigmoidal functions and we do not expect their exact shape to be easily determined from experiments or otherwise.

We assume that the tumor size dynamics are determined by availability of oxygen and nutrients. The amount of nutrients delivered to the tumor is proportional to the volume of blood vessels feeding the tumor, whether inside the tumor or in its close vicinity. To take this into account we use vessel density which may relate to immature vessels, mature vessels, or the total of both, denoted by E_1, E_2 or E, respectively. The vessel density is calculated by dividing the corresponding vessel volume by the tumor size $E = \frac{V}{N}$. To simplify our models we assume that vessel wall permeability (perfusion) is the same for immature and mature vessels. For the tumor size dynamics in all our models we assume the Malthusian law determined by

$$\dot{N} = f_1(E)N(t) \tag{1}$$

where f_1 is an increasing sigmoid function capturing the processes of cell proliferation and death:

$$f_1(E = 0) < 0, \lim_{x\to\infty} f_1(x) > 0. \tag{2}$$

For dynamics of protein (growth factor) compartments we assume that proteins are produced by tumor cells or immature vessels, and degraded by an intrinsic clearance process. Elaboration of the clearance process will be discussed later, suggesting the introduction of additional consuming elements, such as the forming vessels, into the model.

we assume that the dynamics of vessel compartments are a superposition of four processes, some are contrasting some of the others: formation of immature vessels, regression of immature vessels, maturation of immature vessels and destabilization of mature vessels into immature vessels. We assume that these four processes are driven by sigmoid like responses, as described above, depending on specified proteins. These proteins are the stimuli mentioned earlier as the effectors of these functions.

3. **Mathematical background on Hopf points.** When interested in periodic or quasi-periodic behavior of a dynamical system, Hopf points are the points which are first to be considered. In this section we review the basic facts about Hopf points and Hopf bifurcations (see [9], 8.3). Suppose we have a system of autonomous ODEs

$$\dot{v} = F(v, \mu) \tag{3}$$

where $v \in R^n$ is the vector of variables $(v_1, ..., v_n)$ and $\mu \in R^m$ is the vector of parameters. We say that an ordered pair $(v_0, \mu_0) \in R^n \times R^m$ is a *Hopf point*, provided there exists $\varepsilon > 0$ such that there is a smooth function (*associated curve*) $\varphi : [-\varepsilon, \varepsilon] \to R^n \times R^m, \varphi(\xi) = (v_\xi, \mu_\xi))$ satisfying properties

- $\varphi(0) = (v_0, \mu_0)$
- $F(\varphi(\xi)) = 0$ for all $\xi \in [-\varepsilon, \varepsilon]$
- The linearization matrix (see below) at (v_ξ, μ_ξ) has at least one pair of conjugate complex eigenvalues $\alpha(\xi)+\beta(\xi)i$ for all $\xi \in [-\varepsilon, \varepsilon]$ with $\alpha(0) = 0, \alpha'(0) \neq 0, \beta(0) \neq 0$
- All other eigenvalues of the linearization matrix except $\pm\beta(0)i$ have nonzero real parts

Technically finding Hopf points for an ODE dynamical system with smooth right hand side functions amounts to finding all fixed points (depending on system parameters), finding roots of the characteristic polynomial of the characteristic matrix $M - \lambda I$ where $M = \{\frac{\partial F_i}{\partial v_j}|_{v=v_0}\}_{i=1,j=1}^{n,n}$ is the matrix of the linearized system at a fixed point, finding conditions on parameters under which there is exactly one pair of pure imaginary eigenvalues, showing that the fixed point manifold intersects the pure imaginary eigenvalue existence manifold and finding an associated curve. Eigenvalues are just the roots of the characteristic equation

$$\det(M - \lambda I) = 0. \tag{4}$$

Now, suppose we have a dynamical system with a constant time-delay system with delay vector $\tau = (\tau_1, ..., \tau_k)$. We write x_{τ_i} instead of $x(t-\tau_i)$ for any system variable x and denote the vector $(v_{1\tau_i}, ..., v_{n\tau_i})$ by v_{τ_i}. In this notation we have a system

$$\dot{v} = F(v, v_{\tau_1}, ..., v_{\tau_k}, \mu, \tau) \tag{5}$$

In this case, to find Hopf points instead of solving equation (4) we must solve with respect to λ the characteristic equation

$$\det(\tilde{M}) = 0. \tag{6}$$

where the characteristic matrix

$$\tilde{M} = M + \sum_{i=1}^{k} M_{\tau_i} e^{-\lambda\tau_i} - \lambda I \tag{7}$$

with

$$M_{\tau_l} = \{\frac{\partial F_i}{\partial v_{j\tau_l}}|_{v=v_0}\}_{i=1,j=1}^{n,n}. \tag{8}$$

Assuming smoothness and genericity of our systems we will consider the existence of a Hopf point proven if there is a set of parameters for which the characteristic equation has a pure imaginary solution. Clearly we are interested in investigating fixed points having positive tumor size. For more details about time-delay dynamical systems see [15].

4. **Three dimensional models.**

4.1. **A three-dimensional model with no time-delay.** The simplest modelling option presented merely captures the three independent variables mentioned earlier-tumor size N, total vessel volume V and the amount of protein P. The only thing that we assume about the protein is that it drives the vessel formation or regression in a sigmoidal way. The rate of change of N is determined by a Malthusian law sigmoidally depending on E (representing EVD, as defined earlier). The protein P is produced by the tumor at a rate sigmoidally dependent on E and is decaying at a constant positive rate δ. The rate of change of the vessel volume V is also sigmoidally driven by the protein. Thus we have the system

$$\begin{cases} \dot{N} = f_1(E)N \\ \dot{P} = f_2(E)N - \delta P \\ \dot{V} = f_3(P)V \end{cases} \tag{9}$$

where

- f_1 is the tumor cells proliferation rate; it is an increasing function of E and satisfies (2).
- f_2 is the protein production rate; it is a decreasing function of E and satisfies

$$f_2(x) > 0, \lim_{x\to\infty} f_2(x) = 0. \tag{10}$$

- f_3 is the vessel growth rate; it is an increasing function of P and satisfies

$$f_3(P = 0) < 0, \lim_{x\to\infty} f_3(x) > 0. \tag{11}$$

To simplify the analysis we make a substitution of variables $V \to E$ and get a system

$$\begin{cases} \dot{N} = f_1(E)N \\ \dot{P} = f_2(E)N - \delta P \\ \dot{E} = f_3(P)E - f_1(E)E. \end{cases} \tag{12}$$

For each set of parameters which determine f_1, f_2, f_3, the model has one fixed point $Q^{(1)} = (N^{(1)}, P^{(1)}, E^{(1)})$ with $N^{(1)} > 0$, given by $f_1(E^{(1)}) = 0, f_3(P^{(1)}) = 0, N^{(1)} = \frac{\delta P^{(1)}}{f_2(E^{(1)})}$.
We claim that there are no Hopf bifurcation points among this family of steady states.
Here is an explanation: The matrix of the system linearized at such a point is

$$M = \begin{pmatrix} 0 & 0 & f_1'(E^{(1)})N^{(1)} \\ f_2(E^{(1)}) & -\delta & f_2'(E^{(1)})N^{(1)} \\ 0 & f_3'(P^{(1)})E^{(1)} & -f_1'(E^{(1)})E^{(1)} \end{pmatrix} = \begin{pmatrix} 0 & 0 & a'N^{(1)} \\ b & -\delta & -b'N^{(1)} \\ 0 & c'E^{(1)} & -a'E^{(1)} \end{pmatrix} \tag{13}$$

where all the new parameters
$a' = f_1'(E^{(1)}), b = f_2(E^{(1)}), b' = -f_2'(E^{(1)}), c' = f_3'(P^{(1)})$
are positive. We calculate the characteristic equation

$$\det(M - \lambda I) = -(\lambda^3 + \lambda^2(a'E^{(1)} + \delta) + \lambda(a'\delta E^{(1)} + b'c'N^{(1)}E^{(1)}) - (a'bc'N^{(1)}E^{(1)})). \tag{14}$$

If the cubic polynomial in (14) admits a pair of pure imaginary roots $\pm Ai, A \in \mathbf{R}$, then it has the form

$$-(\lambda^2 + A^2)(\lambda + B) = -(\lambda^3 + B\lambda^2 + A^2\lambda + A^2B) \tag{15}$$

for some $B \in \mathbf{R}$. Since the coefficients satisfy $-(a'bc'N^{(1)}E^{(1)}) < 0$, $a'E^{(1)} + \delta > 0$ and $a'\delta E^{(1)} + b'c'N^{(1)}E^{(1)} > 0$, we have that the characteristic polynomial cannot have pure imaginary roots and thus there are no Hopf points with $N \neq 0$ in (12).

4.2. **A model with protein consumption.** We can modify the model assuming that the protein is additionally consumed by growing vessels. Thus, for the dynamics of the protein compartment we suggest

$$\dot{P} = f_2(E)N - \delta P - f_c(\dot{V})P \tag{16}$$

in the second equation of (9), where

$$f_c(x) > 0, \lim_{x \to +\infty} f_c(x) > 0, f_c(0) \approx 0. \tag{17}$$

The system in this case is given by

$$\begin{cases} \dot{N} = f_1(E)N \\ \dot{P} = f_2(E)N - \delta P - f_c(f_3(P)EN)P \\ \dot{E} = f_3(P)E - f_1(E)E. \end{cases} \tag{18}$$

The functions f_1, f_2, f_3 have the same properties as in the model (9). For each set of parameters which determine f_1, f_2, f_3, the model has one fixed point $Q_c^{(1)} = (N_c^{(1)}, P^{(1)}, E^{(1)})$ with $N_c^{(1)} > 0$, given by $f_1(E^{(1)}) = 0, f_3(P^{(1)}) = 0, N_c^{(1)} = \frac{[\delta + f_c(0)]P^{(1)}}{f_2(E^{(1)})}$.

The corresponding matrix for this point is

$$M = \begin{pmatrix} 0 & 0 & f_1'(E^{(1)})N_c^{(1)} \\ f_2(E^{(1)}) & -\delta - f_c(0) - f_c'(0)f_3'(P^{(1)})N_c^{(1)}P^{(1)}E^{(1)} & f_2'(E^{(1)})N_c^{(1)} \\ 0 & f_3'(P^{(1)})E^{(1)} & -f_1'(E^{(1)})E^{(1)} \end{pmatrix}$$
$$= \begin{pmatrix} 0 & 0 & a'N_c^{(1)} \\ b & -\delta - d - c'd'N_c^{(1)}P^{(1)}E^{(1)} & -b'N_c^{(1)} \\ 0 & c'E^{(1)} & -a'E^{(1)} \end{pmatrix} \tag{19}$$

where all the parameters $a', b, b', c', d, d', N, P, E$ are positive. The characteristic equation in this case does not have pure imaginary roots for the same reason as in the case (14) and therefore there are no Hopf points in this model.

4.3. **Models with time-delays.** Two time-delays are introduced in (12): τ_1 in the proliferation/death response to stimuli and τ_2 in the vessel formation/regression response to stimuli.
Let $E_{\tau_1} = E(t-\tau_1), P_{\tau_2} = P(t-\tau_2)$, then system (12) is modified, yielding:

$$\begin{cases} \dot{N} = f_1(E_{\tau_1})N \\ \dot{P} = f_2(E)N - \delta P \\ \dot{E} = f_3(P_{\tau_2})E - f_1(E_{\tau_1}))E. \end{cases} \tag{20}$$

This system has the same fixed points as (12). Again we are only interested in the family of fixed points $Q^{(1)}$. The characteristic equation in this case is

$$\det(\tilde{M}^{(1)}) = \det(M + M_{\tau_1}e^{-\lambda\tau_1} + M_{\tau_2}e^{-\lambda\tau_2} - \lambda I) = 0 \tag{21}$$

where

$$M = \begin{pmatrix} 0 & 0 & 0 \\ f_2(E^{(1)}) & -\delta & f_2'(E^{(1)})N^{(1)} \\ 0 & 0 & 0 \end{pmatrix},$$

$$M_{\tau_1} = \begin{pmatrix} 0 & 0 & f_1'(E^{(1)})N^{(1)} \\ 0 & 0 & 0 \\ 0 & 0 & -f_1'(E^{(1)})E^{(1)} \end{pmatrix}, \tag{22}$$

$$M_{\tau_2} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & f_3'(P^{(1)})E^{(1)} & 0 \end{pmatrix}.$$

Computing the determinant of

$$\tilde{M}^{(1)} = \begin{pmatrix} -\lambda & 0 & a'N^{(1)}e^{-\lambda\tau_1} \\ b & -\delta-\lambda & -b'N^{(1)} \\ 0 & c'E^{(1)}e^{-\lambda\tau_2} & -a'E^{(1)}e^{-\lambda\tau_1} - \lambda \end{pmatrix} \tag{23}$$

we get the equation

$$\begin{aligned} &\lambda^3 + e^{-\lambda\tau_1}\lambda^2(a'E^{(1)}) + \lambda^2\delta + e^{-\lambda\tau_1}\lambda(\delta a'E^{(1)}) + \\ &+e^{-\lambda\tau_2}\lambda(b'c'E^{(1)}N^{(1)}) - e^{-\lambda(\tau_1+\tau_2)}(a'bc'N^{(1)}E^{(1)}) = 0. \end{aligned} \tag{24}$$

After renaming the parameters we get the equation

$$\lambda^3 + c_1\lambda^2e^{-\lambda\tau_1} + \delta\lambda^2 + (c_1\delta)\lambda e^{-\lambda\tau_1} + c_2\lambda e^{-\lambda\tau_2} - c_3e^{-\lambda(\tau_1+\tau_2)} = 0 \tag{25}$$

where $\{c_1, c_2, c_3\}$ are positive parameters which are independent of the fixed point and of each other.
Our goal is to manipulate these parameters so as to find $\lambda = iy, y \in \mathbf{R}$ satisfying (25).
Put $\lambda = iy$ in (25):

$$(iy)^3 + c_1(iy)^2e^{-iy\tau_1} + \delta(iy)^2 + (c_1\delta)iye^{-iy\tau_1} + c_2iye^{-iy\tau_2} - c_3e^{-iy(\tau_1+\tau_2)} = 0. \tag{26}$$

We regard (26) as a linear system of two equations (real and imaginary parts) in the variables c_1, c_2, c_3. It turns out[1] that if at least one of the time-delays τ_1, τ_2 is positive then we can find an appropriate $y \in \mathbf{R}$ such that c_1, c_2, c_3 are all positive. We conclude that for every $(\tau_1, \tau_2) \neq (0,0)$ the family $Q^{(1)}$ contains Hopf points.

[1]The entire argument, separately dealing with four cases according to $\frac{\tau_1}{\tau_2}$, is not included.

5. **Five dimensional models.** To make our models more elaborate and realistic, we introduce more compartments representing more complex vascularity and protein effects. First, the inclusive representation of vessels volume V is replaced by separate descriptions of the immature and mature vessel volumes denoted by V_1 and V_2, respectively. The values of either vessel subpopulation will be separately analyzed. Hence, the model allows both maturation of immature vessels and destabilization of mature vessels. Secondly, the general term protein, denoted P, is now replaced by two specific proteins namely $VEGF$, denoted P_1 and $Ang1$, denoted P_2. We assume that $VEGF$ is produced by the tumor at a rate sigmoidally dependent on the vessel density and decays at a constant rate δ_1, and that $Ang1$ is produced by the tumor at a constant rate α and decays at a constant rate δ_2. Note that another growth factor, $Ang2$, is not modelled here as an additional dimension. Rather, it is assumed to exist in a constant amount. Hence, it is represented as one of the constant parameters wherever relevant in the functions f_1, f_2, f_3. Let us also introduce the characteristic time-delays as follows: τ_1 for tumor proliferation and death, τ_2 for immature vessel formation and regression, and τ_3 for mature vessel destabilization. We get the system:

$$\begin{cases} \dot{N} = f_1(E_{\tau_1})N \\ \dot{P}_1 = f_2(E)N - \delta_1 P_1 \\ \dot{P}_2 = \alpha N - \delta_2 P_2 \\ \dot{V}_1 = f_3(P_{1\tau 2})V_1 - f_4(P_2)V_1 + f_5(P_{2\tau_3})V_2 \\ \dot{V}_2 = f_4(P_2)V_1 - f_5(P_{2\tau_3})V_2 \end{cases} \tag{27}$$

where f_1, f_2, f_3 satisfy (2,10,11) and

- f_4 is the maturation rate, it is a positive increasing function of P_2.
- f_5 is the destabilization rate, it is a positive decreasing function of P_2 and satisfies

$$\lim_{x\to\infty} f_5(x) = 0. \tag{28}$$

After making the substitutions $V_i \to E_i = \frac{V_i}{N}$ and $E_2 \to E = E_1 + E_2$ we get the system

$$\begin{cases} \dot{N} = f_1(E_{\tau_1})N \\ \dot{P}_1 = f_2(E)N - \delta_1 P_1 \\ \dot{E} = f_3(P_{1\tau_2})E_1 - f_1(E_{\tau_1})E \\ \dot{P}_2 = \alpha N - \delta_2 P_2 \\ \dot{E}_1 = f_3(P_{1\tau_2})E_1 - f_4(P_2)E_1 + f_5(P_{2\tau_3})(E - E_1) - f_1(E_{\tau_1})E_1. \end{cases} \tag{29}$$

For each set of parameters which determine f_1, f_2, f_3, f_4, f_5, the model has one fixed point $Q^{(2)} = (N^{(2)}, P_1^{(2)}, E^{(2)}, P_2^{(2)}, E_1^{(2)})$ with $N^{(2)} > 0$ that is given by the conditions $f_1(E^{(2)}) = 0, f_3(P_1^{(2)}) = 0, N^{(2)} = \frac{\delta_1 P_1^{(2)}}{f_2(E^{(2)})}, P_2^{(2)} = \frac{\alpha\delta_1 P_1^{(2)}}{\delta_2 f_2(E^{(2)})}, E_1^{(2)} = \frac{f_5(P_2^{(2)})E^{(2)}}{f_4(P_2^{(2)})+f_5(P_2^{(2)})}$.
The characteristic equation at $Q^{(2)}$ is

$$\det(\tilde{M}^{(2)}) = 0 \tag{30}$$

where

$$\tilde{M}^{(2)} = \begin{pmatrix} -\lambda & 0 & a'N^{(2)}e^{-\lambda\tau_1} & 0 & 0 \\ b & -\delta_1-\lambda & -b'N^{(2)} & 0 & 0 \\ 0 & c'E_1^{(2)}e^{-\lambda\tau_2} & -a'E^{(2)}e^{-\lambda\tau_1}-\lambda & 0 & 0 \\ * & * & * & -\delta_2-\lambda & 0 \\ * & * & * & * & -d-e-\lambda \end{pmatrix}. \quad (31)$$

From the block structure we have that

$$\det(\tilde{M}^{(2)}) = (\lambda+e+d)(\lambda+\delta_2)g(\lambda) = 0 \quad (32)$$

where $g(\lambda)$ has the same structure as $\det(\tilde{M^{(1)}})$ in (24) with time-delays τ_1 and τ_2. Note that (32) does not depend on τ_3. Since all the symbols are positive, the conclusion for this model coincides with the one for (20).
We obtain that for every pair $(\tau_1, \tau_2) \neq (0,0)$, there always exist parameter sets such that $Q^{(2)}$ is a Hopf bifurcation point of the system (29).

We summarize the results about the $3-D$ and the $5-D$ models in the following proposition:

Proposition 5.1. *The ODE systems (20) and (29) admit a Hopf bifurcation point if and only if at least one of the time-delays τ_1 or τ_2 is nonzero.*

6. **Conclusion.** Introduced above were several modelling suggestions describing angiogenesis, with or without time-delays. As demonstrated, whenever time-delay was introduced into the tumor proliferation or the neo-vascularization process, Hopf points were found, leading to oscillatory behavior. In contrast, a time-delay in the mature vessels de-stabilization process does not seem to be necessary for the existence of oscillations in the tumor-volume/vessel-density system. This might mean that an appropriate candidate for describing the system in question is the alternative that includes time-delays in the tumor proliferation or angiogenesis process. While it is recognized that time-delay will often elicit Hopf points, here it was shown that the latter were to be found for any angiogenesis model with time-delay. Further mathematical research is warranted for exploring time-delays in the biologically realistic domains in the parameter space. Moreover, recall that for rationalizing the empirical results it was necessary to introduce a significant time-delay between the tumor and vessels processes. This might underline the significance of time-delays in tumor growth dynamics. It seems to us worthwhile to examine these results in the clinical context, that is, to check whether or not one can contain tumor growth by imposing (by use of certain drugs) time-delays in the processes neo-vascularization.

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