MATHEMATICA APPLICANDA
Vol. 46(1) 2018, p. 3–12
doi: 10.14708/ma.v46i1.6383

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Mathematical model of endothelial cell proliferation and maturation

Abstract Blood vessel sprouting (angiogenesis) is one of the hallmarks of cancer. Better quantitative understanding of this process would allow more effective antiangiogenic therapies to be developed. It has been hypothesised that not only the number of endothelial cells, but also the quality of the vasculature play an important role in how chemo- and radiotherapies are delivered to tumour site. Hence in this study a minimally-parametrised mathematical model of endothelial cell proliferation and maturation is developed. Endothelial cells are subdivided into two compartments – mature and immature (or proliferating). The cells are assumed to undergo a self-mediated maturation, while loss of blood vessel quality is mediated by an external growth factor (here VEGF). The model is fitted to experimental data. The model shows how inhibition of VEGF results in better quality vasculature and slower proliferation.

2010 Mathematics Subject Classification: Primary: 92C50; Secondary: 37N25, 92B05.

Key words and phrases: Angiogenesis, mathematical modelling, tumour growth, vascular endothelial growth factor.

1. Introduction Ever since over 40 years ago Folkman postulated that inhibition of tumour growth is possible by cutting off its blood supply, the idea of therapies which target tumour vasculature has been investigated in numerous studies [2, 6]. The initial enthusiasm for antiangiogenic therapy (i.e. one aiming to inhibit tumour vasculature) was met with somehow discouraging experimental results which suggested that in many cases antiangiogenic therapy by itself cannot effectively inhibit tumour growth. Poor results of antiangiogenic therapy may be attributed to the fact that endothelial cells respond to a variety of stimuli – such as Vascular Endothelial Growth Factor (VEGF), basic fibroblast growth factor or epidermal growth factor – so that blocking one pathway may not be enough.

The tumour-induced vasculature is lacking in pericyte coverage, has many loops and irregularities which impair blood flow. Jain et al [4] observed that inhibiting proangiogenic factors restores the pro/anti-angiogenic balance, which

* Piotr Bajger and Mariusz Bodzioch were supported by Polish National Science Centre grant 2016/23/N/ST1/01178.

in turn results in the normalisation of the abnormal network and increases its quality.

Vascular normalisation has crucial implications for effective therapy scheduling. In theory, blood vessel normalisation post anti-angiogenic therapy could improve drug or radiotherapy delivery to the tumour site. As combination of therapies would require careful timing, tumour growth under angiogenic signalling has been a subject of mathematical modelling. A classic example is the well-studied model of Hahnfeldt et al. [3]. Such models usually consider only the size of the vasculature and those addressing vascular quality either require tens of parameters [1] or adopt a purely phenomenological approach [5].

Therefore this study attempts to formulate a mathematical model (a system of ordinary differential equations) describing endothelial cell growth with a particular emphasis on blood vessel normalisation and its potential therapeutic implications.

2. Methods

2.1. Model To model the process of vascular normalization we subdivide the endothelial cell population into two categories: mature (M) and proliferating (P). The mature cells M are precisely those which form a normalised blood vessel network – they are characterised by good pericyte coverage and are tightly attached to each other. The proliferating cells P are those which become destabilised due to a pro/anti-angiogenic factor imbalance. They lack pericyte coverage, are irregularly interconnected and induce blood vessel sprouting (hence proliferation) thus forming an immature, abnormal network. [4]

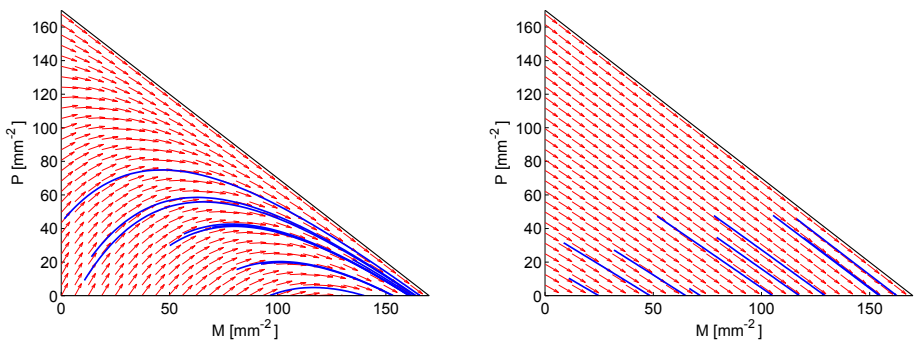
We postulate that the endothelial cell proliferation/maturation dynamics is governed by the following system of ordinary differential equations:

$$\begin{aligned} \dot{M} &= aP - bv(t)M, & M(0) &= M_0, \\ \dot{P} &= -aP + bv(t)M + cP\left(1 - \frac{M+P}{K}\right), & P(0) &= P_0, \end{aligned} \tag{1}$$

where a is the normalization rate, b is the rate at which the blood vessels become abnormal in the presence of $v(t)$ molecules of a generic proangiogenic growth factor, c is the growth rate of abnormal vessels, K is the overall carrying capacity.

2.2. Properties Let us observe first that if $M + P = K$, then $\frac{d}{dt}(M+P) = cP\left(1 - \frac{M+P}{K}\right) = 0$, so that the $M + P = K$ line is invariant under System (1). Consequently, the triangle $\Omega = \{(M, P) : M \geq 0, P \geq 0, M + P \leq K\}$ is invariant under System (1) as well. We will henceforth restrict our domain of interest to Ω.

2.2.1. Growth factor absent Before we consider the action of a growth factor on the vasculature, let us examine the model properties when no growth factor is present, i.e. $v(t) \equiv 0$. In this case there is a continuum of non-isolated steady states of the form $(M_0, 0)$. This is reasonable from a biological point of view – in the absence of proangiogenic factors mature vascular network remains in equilibrium. We may examine the behaviour of the system further by means of a phase diagram, see Figure 1. It may be noted that cases $c > a$ and $c < a$ are qualitatively different. If $a > c$, then for low cell numbers the immature cells proliferate more quickly than maturate. This leads to a significant increase in the overall number of cells. On the other hand, if $a < c$ then the immature cells become mature more quickly than they proliferate and the population stabilises as all cells quickly become mature.



(a) (b)

Figure 1: Phase portraits for System (1) with $v(t) \equiv 0$ in the phase plane (M, P) for (a) $c > a$ and (b) $c < a$.

In fact if $v \equiv 0$, time may be eliminated from System (1) and P may be found explicitly as a function of M. Indeed, notice that

$$\frac{\dot{P}}{\dot{M}} = \frac{dP}{dM} = -1 + \frac{c}{a}\left(1 - \frac{M+P}{K}\right)$$

is a linear first order non-homogeneous equation which may be solved analytically (e.g. by an integrating factor). We find that

$$P(M) = K - M + (P_0 + M_0 - K)\exp\left(\frac{c}{aK}(M_0 - M)\right).$$

It is clear from the phase diagram that $P(t) \to 0$ as $t \to \infty$. By considering a limit $t \to \infty$ it follows that $M_\infty = \lim_{t\to\infty} M(t)$ is the unique solution to

$$M_\infty - K = (P_0 + M_0 - K)\exp\left(\frac{c}{aK}(M_0 - M_\infty)\right)$$

lying in the range $[M_0, K]$. To see that it does indeed exist uniquely it is enough to notice the following properties: (i) LHS (left-hand side) is an increasing function of M_∞, while RHS (right-hand side) is a decreasing function

of M_∞, (ii) at $M_\infty = M_0$ we have $LHS < RHS$, and (iii) at $M_\infty = K$ we have $LHS = 0 > RHS$.

2.2.2. Growth factor present Let us now suppose that $v(t) = v = \text{const} > 0$. System (1) has now two steady states:

$$S_0 = (0,0) \quad \text{and} \quad S_1 = (M^*, P^*) = \left(\frac{a}{a+bv} K, \frac{bv}{a+bv} K \right).$$

It can be shown that S_0 is a saddle point, while S_1 is a stable node. In fact by noting that

$$\frac{d(M+P)}{dt} = cP \left(1 - \frac{P+M}{K} \right) > 0$$

for all (M, P) in Ω we may also exclude periodic solutions. Hence by Poincaré-Bendixson theorem, any trajectory starting in Ω will tend to S_1. Phase diagrams are depicted in Fig. 2. A qualitative difference between the $c > a$ and $c < a$ cases is seen again (see Section 2.2.1). If $c > a$, immature cells initially proliferate more quickly than they maturate, hence the network destabilises before reaching the stationary state. If $c < a$, the destabilisation effect is less visible and the immature/mature ratio is roughly constant.

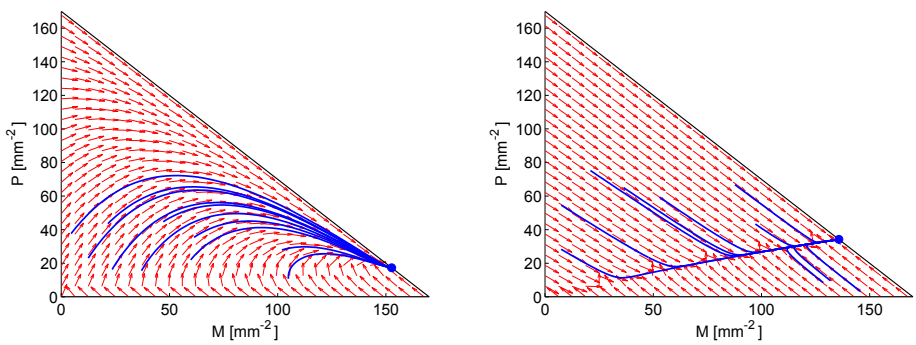


Figure 2: Phase portraits for Eqs. (1) in the phase plane (M, P) for $c > a$ (a) and $c < a$ (b).

Note that the total size of the vasculature at the steady state S_1 is always equal to K and does not depend on other model parameters. In particular it does not depend on the growth factor concentration. However, the ratio $Q := \frac{M^*}{P^*+M^*} = \frac{a}{bv+a}$ at the steady state, which can be considered a measure of the quality of the vasculature, decreases as the growth factor concentration increases.

2.3. Model fitting and validation In this section we examine the capability of System (1) to reproduce experimental data on *in vitro* endothelial cell growth. We will use experimental data from Wolfe's et al. [7] study

on the effects of proangiogenic factors on endothelial cell proliferation and tube formation. In their experiment Wolfe et al. measured the tube length formed by human umbilical vein endothelial cells (HUVEC) under different concentrations of VEGF (among others).

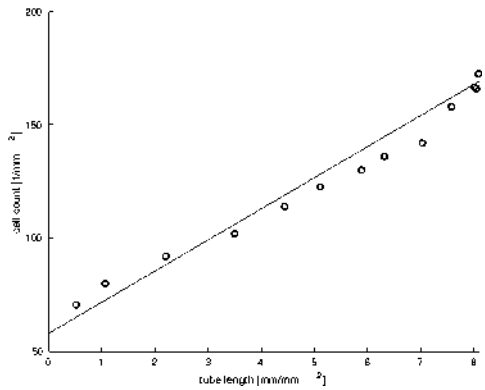


Figure 3: Experimental cell count plotted against tube length (circles) and the best linear fit (solid line). The linear relation obtained is used subsequently to infer cell count from tube length. All data (HUVEC under $4ng/mL$ VEGF) obtained from [7].

2.3.1. Data preparation The data reported in [7] is the total tube length formed by HUVEC (in mm/mm^2). The dependent variables in System (1) are the cell counts, hence the first step in our analysis is to align the experimental data units with the model described in this study. Fortunately Figure 3 in [7] shows both cell count ($1/mm^2$) and tube length (mm/mm^2) developing over 14 days for a set of cultures. Figure 3 shows the cell count plotted against the tube length. Each point corresponds to measurements taken at a single point in time. Only points with tube length greater than $0.5mm/mm^2$ were considered, as it corresponds to the minimum tube length of $0.34mm/mm^2$ used in the fitting process. The graph suggests that the relation between the two quantities is linear, so that we postulate that the tube length L and cell count C are related by

$$C = \alpha L + \beta, \tag{2}$$

with $\alpha = 13.79[1/mm]$ and $\beta = 58.17[1/mm^2]$. The trend line is shown in Figure 3. The parameters α and β were linearly regressed and estimated by the least squares method. With these parameters, the model accounts for $R^2 = 97\%$ of data variation. We postulate this linear relation to be valid at least for $L > 0.5mm/mm^2$.

Relation (2) allows us to estimate the initial cell density from the initial tube length of $0.34[mm/mm^2]$. The corresponding cell count is $M_0 = 62.84[1/mm^2]$, which is used as an initial condition for System (1) throughout

this study. At the same time we assume that no HUVEC cells were initially proliferating, i.e. $P_0 = 0$.

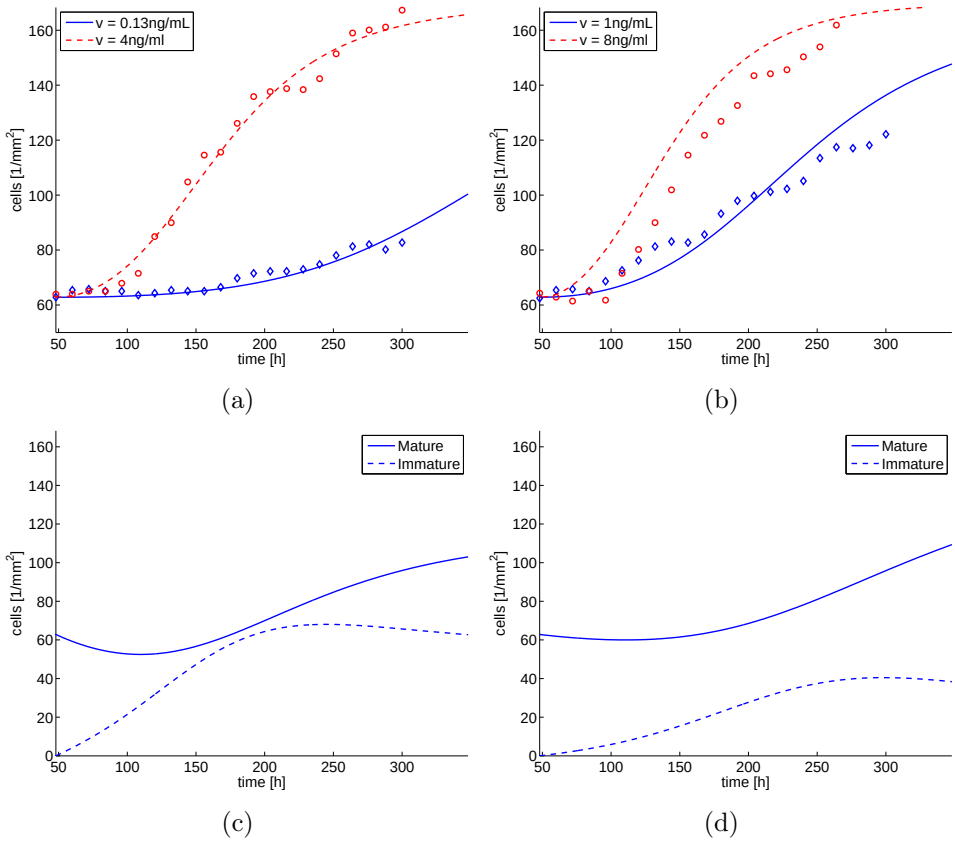


Figure 4: Simulated total number of endothelial cells (M+P) plotted with the corresponding experimental learning datasets (a) and validation datasets (b). A breakdown of the total number of HUVEC intro mature and immature is shown for $v = 4ng/mL$ (c) and $v = 1ng/mL$ (d).

2.3.2. Fitting Note that all the simulations were performed under a simplifying assumption that $v(t) \equiv \text{const}$. We believe this is justified due to the experimental set-up being designed to keep the VEGF concentration stable by frequent medium refreshing. We fitted the output of System (1) to two sets of experimental data on HUVEC cells tube formation under $0.13ng/mL$ and $4ng/mL$ VEGF from [7]. The fitting was performed using the weighted least squares method: given a set of measurements $C_1, C_2, ..., C_k$ at times $t_1, ..., t_k$ we aimed to minimize $J(a, b, c, K; v) = \sum_{i=1}^{k} \frac{\left(M(t_k;v)+P(t_k;v)-C_k\right)^2}{C_k^2}$, where the dependence of M and P on a, b, c, K is implicit.

Wolfe et al. concluded that there is little difference in HUVEC proliferation between VEGF concentration of 4 and 8 milligrams per milliliter, thus

Symbol	Value	Unit	Role
α	13.79	$1/mm$	Linear coefficient in the cell count vs tube length relation (2)
β	58.17	mm^{-2}	Intercept in the cell count vs tube length relation (2)
a	1.05×10^{-2}	h^{-1}	HUVEC maturation rate
b	1.32×10^{-3}	$ng(mL \times h)^{-1}$	VEGF-induced HUVEC dematuration rate
c	3.67×10^{-2}	h^{-1}	HUVEC growth rate
K	1.70×10^{2}	mm^{-2}	HUVEC carrying capacity
M_0	62.84	mm^{-2}	Initial concentration of mature HUVEC.
P_0	0	mm^{-2}	Initial concentration of proliferating HUVEC.

Table 1: Fitted parameter values used in data preparation and simulations.

suggesting a saturating character of VEGF action. In terms of the theoretical model this is reflected in a dependence of steady state vascular immaturity $1 - Q = \frac{P^*}{M^*+P^*} = \frac{v}{v+a/b}$. This gives us a rough estimate on the ratio a/b, namely we postulate that $a/b = O(1)$ and $a/b < 8$. The latter condition was incorporated as a constraint in the fitting process.

The results of the fitting are shown in Figure 4a, while the values of the fitted parameters are listed in Table 1. For the purpose of validation, the parameter values obtained in the fitting were tested against the two remaining datasets, as shown in Figure 4b. In Figures 4c and 4d the simulated mature and immature HUVEC cells are shown separately.

3. Numerical results Having fitted and validated the model against experimental data, we are now in position to utilise the model's predicting capability.

As noted in the previous sections, any trajectory with $v > 0$ will eventually become arbitrarily close to the positive steady state $S_1 = \left(\frac{a}{a+bv}K, \frac{bv}{a+bv}K\right)$. In this sense, any therapy aimed at reduction of the concentration or binding of VEGF will never prevent the abnormal vasculature from developing. The VEGF concentration, however, has an effect on the time needed to reach the steady state. Figure 5a shows the time $\tau(\omega, v)$ needed by the total number of cells to reach size ωK (for selected values of $\omega \in (0, 1]$) given a VEGF concentration v, i.e.

$$\tau(\omega, v) = \inf_{t \geq 0} \{t : M(t) + P(t) \geq \omega K\}.$$

It it is visible that the time needed to reach the steady state decreases as the VEGF concentration increases. Thus inhibition of VEGF would be a reasonable strategy to delay, although not completely stop, angiogenesis.

Even though VEGF concentration effectively increases the overall proliferation rate of HUVEC population, the qualitative nature of the network changes too. Figure 5c shows how quality of the vasculature (measured as the ratio of mature cells to the total number of cells) varies with the VEGF concentration as the HUVEC cells proliferate towards the carrying capacity, i.e.:

$$Q(\omega, v) = \frac{M(\tau(\omega,v))}{M(\tau(\omega,v))+P(\tau(\omega,v))}.$$

Increasing VEGF concentration generally has a destabilising effect on the vasculature and decreases its quality, as shown in Fig. 5b. Simulation results indicate however, that if the snapshot is taken when the vasculature reaches a certain size, the relationship is not obvious (Fig. 5c).

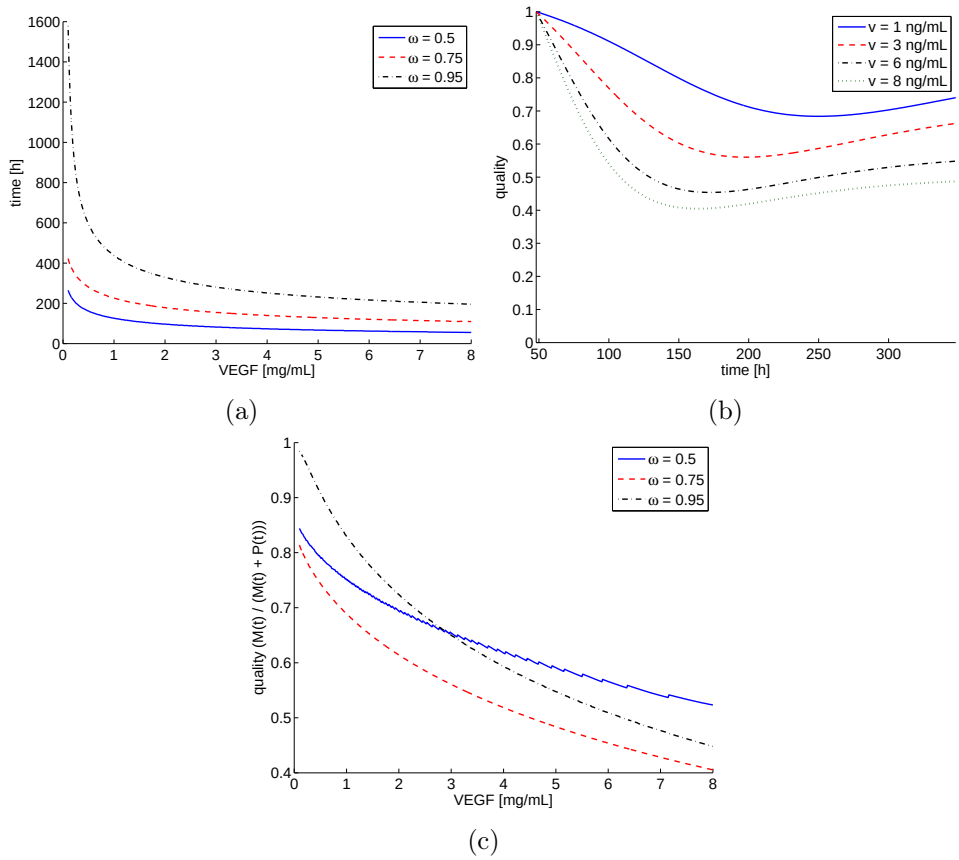


Figure 5: (a) Simulated time τ needed by HUVEC to reach size ωK as a function of VEGF concentration. (b) Simulated quality Q of the vasculature over time for different values of VEGF concentration. (c) Simulated quality Q of the vasculature at the point when its total size is equal to ωK as a function of VEGF concentration.

4. Discussion An ordinary differential equation mathematical model describing endothelial cells maturation/dematuration and proliferation was

described in this study. The model was fit to the experimental data and the effects of VEGF concentration on the vasculature quality were examined.

The model correctly captures some key quantities relevant to endothelial cell proliferation. It allows for prediction of not only the growth rate, but also, to some extent, the quality of the vasculature, measured as the ratio of mature to total number of endothelial cells. Potential applications of the model include an *in silico* assessment of different kinds of antiangiogenic therapy.

At this point the model suffers from two drawbacks. Firstly, the total size of the vasculature at the globally stable steady state does not depend on the VEGF concentration, which is not in exact agreement with the experimental data. Further study is needed in order to examine whether a modification of the model is necessary to incorporate this feature. Secondly, the VEGF concentration is considered constant, which may be an oversimplification. Extensions of the model would incorporate the actual dynamics of the growth factor (secreted by, for example, hypoxic tumour cells).

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Matematyczny model proliferacji i dojrzewania komórek śródbłonka

Piotr Bajger, Mariusz Bodzioch

Streszczenie Angiogeneza jest jedną z cech charakterystycznych raka. Lepsze ilościowe zrozumienie tego procesu pozwoliłoby na opracowanie skuteczniejszych terapii antyangiogennych. Postawiono hipotezę, że nie tylko liczba komórek śródbłonka, ale także jakość układu naczyniowego, odgrywa ważną rolę w sposobie, w jaki chemio- i radioterapie są dostarczane do guza. W związku z tym rozważamy minimalnie sparametryzowany matematyczny model proliferacji i dojrzewania komórek śródbłonka. Komórki śródbłonka dzielą się na dwa rodzaje — dojrzałe i niedojrzałe (lub proliferujące). Zakłada się, że komórki ulegają samoistnemu dojrzewaniu, podczas gdy utracie jakości naczyń krwionośnych pośredniczy zewnętrzny czynnik wzrostu (tutaj VEGF). Rozważany model został dopasowany do danych eksperymentalnych. Pokazuje on, w jaki sposób hamowanie VEGF prowadzi do lepszej jakości układu naczyniowego i wolniejszej proliferacji.

Klasyfikacja tematyczna AMS (2010): 92C50; 37N25; 92B05.

Słowa kluczowe: Angiogeneza, modelowanie matematyczne, wzrost guza, czynnik wzrostu śródbłonka naczyniowego.



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Communicated by: Urszula Foryś

(Received: 13th of April 2018; revised: 14th of June 2018)