Mathematical analysis of Glioblastoma invasion models from in vitro experiment

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Abstract: Stein et al. proposed a continuum mathematical model describing Glioblastoma invasion observed in their experiments on the patterns of growth and dispersion of U87MG tumour spheroids in a three-dimensional collagen-I gel. They identify and characterise discrete cellular mechanisms underlying invasive cell motility from the experimental data. However in their experiments it is observed microscopically that the U87MG invasive cells often exhibit more complicated and irregular behaviour than expressed by their model. We propose a mathematical model by generalising the radially biased motility term in their model based on the mechanism govering the behaviour of U87MG cell in the experiment. We show a rigorous mathematical analysis of our model and give computer simulations of the experiment based on our mathematical model.

Key–Words: Glioblastoma, 3D invasion, Tumour, radially biased motility, Collagen gel, Mathematical model, Mathematical analysis, Computer simulation, Existence of solution, N-cadherin.

1 Introduction

In 2007 Stein et al. [30] presented results from their experiment where tumour spheroids are grown in three-dimensional collagen gels (cf. [4], [8], [9], [28], [32]). They describe a continuum mathematical model, based on a Swanson's model (see [29]), that allows us to quantitatively interpret the data. Their mathematical model reproduces a characteristic behaviour of the U87MG invasive cells that they have a strong radial directional motility bias away from the spheroid center. Fitting the model to the experimental data it is considered that glioma cells invade in a more biased manner, away from the tumour spheroid and are shed from the spheroid at a great rate, suggesting lower cell-cell adhesion and they specified the extent of invasive cell population. If we follow to their mathematical model, the path of invasive cell should radiate along an invariant direction and at a constant velocity. However it is observed that they often exhibit more complicated and unexpected behaviour, such as greatly turn around or turn back to one's path or so. In order to describe such kind of behaviour of each cell we generalise their mathematical model by extending the radially biased motility term and provide our mathematical model.

The goal of this paper is to better understand the mechanism governing invasive cell behaviour. We show rigorous mathematical analysis of our model and computer simulations of cell motility closer to real trajectories from the experiment than Stein's one, based on our mathematical model.

1.1 Mathematical models

Several mathematical models have been known in the literature for cell invasion ([1]-[3], [5], [10], [35]). In the model for core and invasive cell behaviour by Swanson et al. [29], tumour growth is described by a reaction-diffusion equation:

$$\frac{\partial u}{\partial t} = D\nabla^2 u + gu\left(1 - \frac{u}{u_{max}}\right) \tag{1.1}$$

where cell concentration u moves along undirected, random paths as a function of position and time, cells throughout the tumour are assumed to proliferate at a constant rate g until they reach a limiting density, u_{max}, the constant

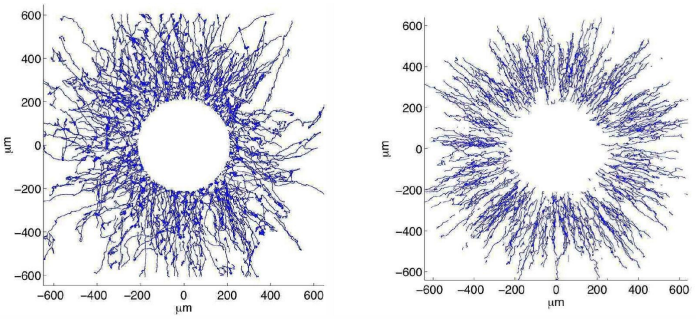
D is the diffusion (undirected motion); the larger D becomes, the more motile the cells. This model assumes spherical symmetry of the multicellular tumour spheroid. The single-population reaction-diffusion model has been used with some success to describe how a tumour responds to chemotherapy and why surgical removal of GBM is usually not effective ([29]). This model is only applicable for tumours that are $> 1mm^3$ and it fails for smaller tumours.

Stein et al. [30] considered that the invasive cells are biased to move away from the center of the tumour spheroid at an average speed, v (cf. [32], [34]). It has been observed that invasive cells may follow to radially directed paths away from the tumour spheroid. The cause of this bias might be due to some attractant in the environment, specified in Remark 1, repulsion from waste products produced by the spheroid, or a realignment of the collagen gel as the cells move. They proposed the following equation for the evolution of the cell population, u

$$\frac{\partial u}{\partial t} = \underbrace{D\nabla^2 u}_{\text{diffusion}} - \underbrace{v\nabla_r \cdot u}_{\text{a radially biased motility}} + \underbrace{s\delta(r - R(t))}_{\text{shedding invasive cells rate}} + \underbrace{gu\left(1 - \frac{u}{u_{max}}\right)}_{\text{proliferation}}. \quad (1.2)$$

The behaviour of invasive cells can be described by four parameters: $\{D, v, s, g\}$. Invasive cells are introduced into the population through shedding from the core surface, s, and proliferation, g. Cell motility is modeled as having an undirected component, D, and a radially biased motile constant, v. In the above equation, δ is the Dirac delta function, r is the spatial coordinate for the radial distance from the tumour center, and $R(t)$ is the radius of the core at time t.

In the experiment of glioma tumour 3D invasion in collagen gel by Stein et al. (cf. [28], [30],[32]), invasive cells with the radially directed motility away from the spheroid center make a progress in the beginning and later they often exhibit more complicated behaviour (see Figure 1(a)). It seems that such complicated behaviour of invasive cells can not be well reproduced by their simulation as in Figure 1(b), because their radially biased motility term of (1.2) is in the *linear* form. In order to describe *nonlinear* paths of cells we need to consider a mathematical model generalised the radially biased component in (1.2) to a *nonlinear* term. Then we give rigorous mathematical analysis of our mathematical model and computer simulation of cell motility based on it.



(a) (b)

Figure 1. (a) Cell trajectories (b) Simulation of cell trajectories, from in vitro experiment of glioma tumour U87MG 3D invasion in collagen-I gel performed by Stein and coworkers in [32] (cf. [28]).

In Figure 1, compared (a) with (b), each path of (b) is seen to be much simpler than (a).

1.2 Mathematical model generalising the term of radially biased motility

Since we especially focus on the behaviour of each dispersing cell leaving from the center the spheroid, neglecting the effect of δ function and proliferation in (1.2), that is, we consider instead of (1.2)

$$\frac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r \cdot u. \quad (1.3)$$

Further we generalise $\nabla_r \cdot u$ to some nonlinear term as follows. For $r = (r_1, \cdots, r_n)$ we have

$$\begin{aligned} \nabla_r \cdot u &= (r_1, \cdots, r_n) \cdot (u_{x_1}, \cdots, u_{x_n}) \\ &= \nabla u \cdot (r_1, \cdots, r_n) = \nabla \cdot u(r_1, \cdots, r_n) \\ &= \nabla \cdot u \nabla(x_1 r_1 + \cdots + x_n r_n), \end{aligned}$$

replacing $(x_1 r_1 + \cdots + x_n r_n)$ by $\log(\alpha + w)$ for a new unknown function w and a non-negative constant α

$$\nabla \cdot (u\nabla \log(\alpha + w)). \quad (1.4)$$

Therefore (1.3) is extended to the following equation.

$$\frac{\partial u}{\partial t} = D\nabla^2 u - \nabla \cdot (u\nabla \log(\alpha + w)). \quad (1.5)$$

In fact, when we put $w = e^{x_1 r_1 + \cdots + x_n r_n} - \alpha$, it is seen that $\log(\alpha + w) = x_1 r_1 + \cdots + x_n r_n$, which

means that (1.5) is a generalisation of (1.3) rigorously. (1.5) is considered in a more general from by Othmer-Stevens [25] which is a continuum model of reinforced random walk where w is called control species and $\log(\alpha + w)$ is a sensitivity function (see Davis [6]). Hence it is seen that (1.5) admits a random walking of the invasive cell along the direction and the velocity indicated by the radially biased component in (1.2). The following system for (1.5) is applied to a understanding of tumour angiogenesis ([2], [24]).

$$(1.6)\begin{cases} \dfrac{\partial u}{\partial t} = D\nabla^2 u - \nabla \cdot \chi_0(u\nabla \log(\alpha + w)) & \\ \qquad\qquad in \quad \Omega \times (0,\infty) & \\ \dfrac{\partial w}{\partial t} = -kuw \qquad in \quad \Omega \times (0,\infty) & \\ \dfrac{\partial}{\partial n}u|_{\partial\Omega} = 0 \qquad on \quad \partial\Omega \times (0,\infty) & \\ u(x,0) = u_0(x) \qquad in \quad \Omega & \end{cases}$$

where D is a positive constant, Ω is a bounded domain in R^n and $\partial\Omega$ is a smooth boundary of Ω and n is the outer unit normal vector and χ_0 is a positive constant .

The second equation describes the interaction between each endothelial cell and some attractant. In this paper we assume that such attractant is N-cadherin.

Remark 1. *In fact,it is known that N-cadherin is produced on the surface of invasive tumour cell due to the interaction with collagen-I and it triggers tumour cells migration(see [23]).*

2 Mathematical analysis

In this section we review known mathematical results related to Othmer and Stevens model and Anderson and Chaplain model, which includes (1.6) and play an important role to carry out the computer simulation in the latter.

2.1 Known result

In Kubo [17] and Kubo and Kimura [18] the following initial Neumann-boundary value problems of nonlinear evolution equations is considered (cf. [19]-[22]).

$$(NE)\begin{cases} u_{tt} = D\nabla^2 u_t + \nabla \cdot (\chi(u_t, e^{-u})e^{-u}\nabla u) & \\ \qquad\qquad in \quad \Omega \times (0,\infty) \quad (2.1) & \\ \dfrac{\partial}{\partial n}u|_{\partial\Omega} = 0 \qquad on \quad \partial\Omega \times (0,\infty) \quad (2.2) & \\ u(x,0) = u_0(x), u_t(x,0) = u_1(x) \quad in \quad \Omega \quad (2.3) & \end{cases}$$

Suppose that the following assumption (A) holds.

(A) Let $B_{r+} = B_r \cap R \times R_+$, where B_r is a ball of radius r at 0 in R^2. For any constant $r > 0$ and $(s_1, s_2) \in B_{r+}$ there exist positive constants c_r, c_r' and δ_r such that for a parameter $b > 0$ and any integer $m \geq [n/2] + 3$

$$c_r(b - \delta_r) < \chi(s_1 + b, s_2) \in C^m(R \times R_+), \quad (2.4)$$

$$\sup_{\substack{(s_1,s_2)\in B_{r+} \\ 0\leq k+l\leq m}} |(\partial_{s_1}^k \partial_{s_2}^l \chi)(s_1 + b, s_2)| \leq c_r', \quad (2.5)$$

where we denote $\dfrac{\partial}{\partial s_i} = \partial_{s_i}, i = 1, 2.$

Now let us introduce function spaces. First, $H^l(\Omega)$ denotes the usual Sobolev space $W^{l,2}(\Omega)$ of order l on Ω. For functions $h(x,t)$ and $k(x,t)$ defined in $\Omega \times [0,\infty)$, we put

$$(h,k)(t) = \int_\Omega h(x,t)k(x,t)dx,$$

$$\|h\|_l^2(t) = \sum_{|\beta|\leq l} \|\partial_x^\beta h(\cdot,t)\|_{L^2(\Omega)}^2, \quad (2.6)$$

where $\partial_x = (\partial_{x_1}, \cdots, \partial_{x_n}), \partial_{x_i} = \dfrac{\partial}{\partial x_i}, i = 1, \cdots, n$ and $\beta = (\beta_1, \cdots, \beta_n)$ is a multi-index.

The eigenvalues of $-\Delta$ with the homogeneous Neumann boundary conditions are denoted by $\{\lambda_i | i = 1, 2, \cdots\}$, which are arranged as $0 < \lambda_1 \leq \lambda_2 \leq \cdots \to +\infty$, and $\varphi_i = \varphi_i(x)$ indicates the L^2 normalised eigenfunction corresponding to λ_i.

For a non-negative integer l, we denote by $W^l(\Omega)$ the function space spanned by $\{\varphi_1, \varphi_2, \cdots, \varphi_n, \cdots\}$ in $H^l(\Omega)$. Taking $\lambda_1 \neq 0$ into account, it is noticed that we have $\int_\Omega h(x)dx = 0$ for $h(x) \in W^l(\Omega)$, which enables us to use Poincare's Inequality. Then the following result is obtained in [17] and [18].

Theorem 2. *Assume that (A) holds and* $(h_0(x), h_1(x)) \in W^{m+1}(\Omega) \times W^m(\Omega)$ *for* $h_0(x) = u_0(x) - a$ *and* $h_1(x) = u_1(x) - b$. *For sufficiently*

large a and any $b > 0$ there is a solution $u(x,t)(= a+bt+v(x,t)) \in \bigcap_{i=0}^{1} C^i([0,\infty); H^{m-i}(\Omega))$ to (NE) such that for $\overline{u}_1 = |\Omega|^{-1} \int_\Omega u_1(x)dx$

$$\lim_{t\to\infty} \|u_t(x,t) - \overline{u}_1\|_{m-1} = 0. \tag{2.7}$$

Remark 3. *The above theorem implies that $u(x,t)$ is a classical solution of (NE) and $u_t(x,t) \to b$ as $t \to \infty$. Also this result justifies the computer simulation based on the mathematical model (1.6) shown in section 3.*

2.2 Application to mathematical models

(i) First we apply Theorem 1 to our problem (1.6) following to Levin and Sleeman [24]. Put $\log w(x,t) = -\int_0^t u(x,\tau)d\tau = U(x,t)$ in the second equation of (1.6), then the first two equations of (1.6) are reduced to

$$U_{tt} = D\Delta U_t + \nabla \cdot \left(\frac{\chi_0 e^{-U}}{1+\alpha e^{-U}} U_t \nabla U \right)$$

which is regarded as the same type of equation as (2.1) and satisfies the condition (A). Therefore it is clear that Theorem 1 holds for (1.6) and it implies that there exists a classical solution $u(x,t)$ to (1.6) such that

$$\lim_{t\to\infty} \|u(x,t) - \overline{u}_0\|_{m-1} = 0.$$

(ii) In [25] Othmer and Stevens proposed a parabolic-ODE system arising from reinforced random walks, which is applied to chemotactic aggregation of myxobacteria etc.,

$$P_t = D\Delta P - D\nabla \cdot P\nabla \log \Phi(W), W_t = \pm kWP, \quad in \quad \Omega \times (0,\infty) \tag{2.8}$$

$$P\nabla \left(\log \frac{P}{\Phi(W)} \right) \cdot n = 0, \quad on \quad \partial\Omega \times (0,T) \tag{2.9}$$

$$P(x,0) = P_0(x), W(x,0) = W_0(x) \geq 0, \quad in \quad \Omega \tag{2.10}$$

where the sensitivity function is given by Levin and Sleeman [24] in the form

$$\Phi(W) = \left(\frac{W+\alpha}{W+\beta} \right)^a, \quad \alpha, \beta > 0,$$

the unknown functions $P = P(x,t)$ and $W = W(x,t)$ stand for the particle density of a particular species and the density of local control species, respectively. Levine and Sleeman [24] applied the model for the understanding of tumour angiogenesis. The existence of global solutions of (2.8)-(2.10) are studied (see [14]-[22]) in the same manner as in (i).

We can carry out computer simulations of these models appeared in (i)-(ii) by the rule of reinforced random walk because Othmer-Stevens model is a continuum model of reinforced random walk (see Davis [6]) and based on it Sleeman and Wallis [33]. Since (1.6) is considered as a special case of Othmer-Stevens model, the simulations of the models of (i)-(ii) can be conducted by using the rule of reinforced random walk, which shown in Figures 4 in the next section. As mentioned, the cause of radially biased motility of cells mainly depends on N-cadhelin and in our model we can consider a mechanism deriving invasive cells radially as reinforced randomwalk instead of the linear term deriving radially biased motility of invasive cells in Stein et al. [30].

On the other hand, the simulation for a mathematical model of in vitro experiment for endothelial cell migration is given by [27], [31] in the similar way.

3 Computer simulation

In this sections we carry out computer simulation based on our mathematical model by reinforced random walk according to Sleeman and Wallis [33] (see [25]), which are shown in Figures 5 and 6.

The following picture in Figure 2 is the image of the 2D projection of the experiment in vitro of glioma tumour 3D invasion in collagen gel performed by Eke and coworkers in [8], which is the same type of experiment as Stein et al. ([30]). For our convenience we choose only seven typical curved paths of each single invasive cell from the experiment and draw them as solid lines marking by (a)-(c) with moderately curved paths, (d)-(g) with g reatly curved trajectries of each single invasive cell on the picture (see Figure 2). In Figure 3 we intend to reproduce the three solid lines in Figure 2 by using 3D random walk type of simulations based on our mathematical model.
All the computer simulations shown in this section are conducted by Mathematica 8.

We can reproduce (a)-(g) in Figure 2 by computer simulations in Figure 3 based on our

model. It is evidently so closer to the real paths than Stein's type of simulation Figure 1 (b).

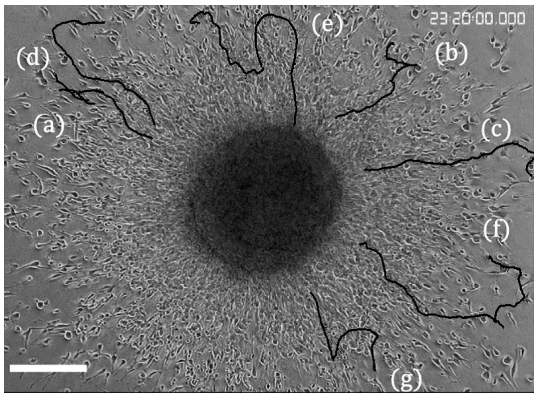


Figure 2. The image of 2D projection to $x-y$ plain of the experiment in vitro of U87MG glioma 3D invasion in collagen gel performed by Eke and coworkers in [8], which is similar to the experiment in [30], [35]. The path (c) indicates that the cell initially radiates and after that turns around greatly. In the path (b) it is observed that the cell changes the direction several times. In the path (a) once the cell arrives at the edge of the extent of invasive cells, it suddenly turns back to one's way and after that moves from the center to the outside again. The paths (d)-(g) are curved more greatly.

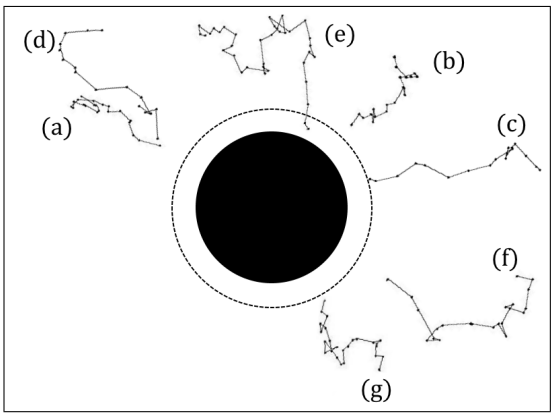


Figure 3. Simulations of the path of each cell corresponding to the solid lines (a)- (g) in Figure 2 respectively..

4 Conclusion

The data of the experiment provides clear evidence that the tumour spheroid cells move away from it at a constant rate initially in the radial direction and after that the radial velocity bias decrease. It seems to be important to gain the understanding of the mechanism of invasion in these in vitro experiments so that their usefulness in understanding the in vitro situation can be understood. However in the mathematical model by Stein et al. [30] the radially biased component implies that the cell motility with a constant velocity and a constant radial direction is quite different from real cell paths observed in the experiment. Supposed that the cause of radial bias is due to some attractant, N-cadhelin, we propose a mathematical model generalised and improved the radially biased motility term of the model of [30] so that it covers more realistic motility as observed in the experiment of Eke et al. [8] or Stein et al. [30] [32]. In fact, we choose some typical paths of U87MG cells in the experiment as shown in Figure 2. We show a rigorous mathematical analysis of our model and give computer simulations corresponding to solid lines in Figure 2 based on our mathematical model, which realise more realistic behaviour of invasive cell than Stein's type of ones.

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References:

[1] Anderson, A. R. A., *Solid tumour invasion: the importance of cell adhesion*, Function and Regulation of Cellular Systems, Birkhäuser Basel, (2004), 379-389.

[2] Anderson, A. R. A. and Chaplain, M. A. J., *Continuous and discrete mathematical models of tumor-induced angiogenesis*, Bulletin of Mathematical Biology, **60(5)** (1998), 857-899.

[3] Anderson, A. R. A. and Chaplain, M. A. J., *A mathematical model for capillary network formation in the absence of endothelial cell proliferation*, Applied Mathematical Letters, **11(3)** (1998), 109-114.

[4] Badiga, A. V., Chetty, C., Kesanakurti, D., Are, D., Gujrati, M., Klopfenstein, J. D., Dinh, D. H. and Rao, J. S., *MMP-2 siRNA inhibits radiation-enhanced invasiveness in glioma cells*, PLoS One, **6(6)** (2011), 1-14.

[5] Chaplain, M. A. J. and Anderson, A. R. A., *Modeling the growth and form of capillary networks*, On Growth and Form, Wiley, (1999), 225-249.

[6] Davis, B., *Reinforced random walks*, Probability Theory and Related Fields, **84(2)** (1990), 203-229.

[7] Demuth, T. and Berens, M. E., *Molecular mechanisms of glioma cell migration and invasion*, Journal of Neuro-Oncology, **70(2)** (2004), 217-228.

[8] Eke, I., Storch, K., Kästner, I., Vehlow, A., Faethe, C., Mueller-Klieser, W., Taucher-Scholz, G., Temme, A., Schackert G. and Cordes, N., *Three-dimensional Invasion of Human Glioblastoma Cells Remains Unchanged by X-ray and Carbon Ion Irradiation In Vitro*, International Journal of Radiation Oncology Biology Physics, **84(4)** (2012), 515-523.

[9] Friedl, P. and Alexander, S., *Cancer invasion and the microenvironment: plasticity and reciprocity*, Cell, **147(5)** (2011), 992-1009.

[10] Ferreira, S. C., Martins, M. L. and Vilela, M. J., *Reaction diffusion model for the growth of avascular tumor*, Physical Review E, **65(2)** (2002).

[11] Giese, A., Bjerkvig, R., Berens, M. E. and Westphal, M., *Cost of migration: invasion of malignant gliomas and implications for treatment*, Journal of Clinical Oncology, **21(8)** (2003), 1624-1636.

[12] Giese, A., Loo, M. A., Tran, N., Haskett, D., Coons, S. W. and Berens, M. E., *Dichotomy of astrocytoma migration and proliferation*, International Journal of Cancer, **67(2)** (1996), 275-282.

[13] Hoelzinger, D. B., Mariani, L., Weis, J., Woyke, T., Berens, T. J., McDonough, W. S., Sloan, A., Coons, S. W. and Berens, M. E., *Gene expression profile of Glioblastoma Multiforme invasive phenotype points to new therapeutic targets*, Neoplasia, **7(1)** (2005), 7-16.

[14] Kubo, A., *A mathematical approach to Othmer-Stevens model*, WSEAS Transactions on Biology and Biomedicine, **1(2)** (2005), 45-50.

[15] Kubo, A., *Qualitative Characterization of Mathematical Models of Tumour Induced Angiogenesis*, WSEAS Transactions on Biology and Biomedicine, **7(3)** (2006), 546-552.

[16] Kubo, A., *Mathematical analysis of some models of tumour growth and simulations*, WSEAS Transactions on Biology and Biomedicine, **2(7)** (2010), 31-40.

[17] Kubo, A., *Nonlinear evolution equations associated with mathematical models*, Discrete and Continous Dynamical Systems supplement 2011, (2011), 881-890.

[18] Kubo, A. and Kimura, K., *Mathematical analysis of tumour invasion model with proliferation*, WSEAS Transaction on Biology and Biomedicine, **11** (2014), 165-173.

[19] Kubo, A. and Suzuki, T., *Asymptotic behaviour of the solution to a parabolic ODE system modeling tumour growth*, Differential and Integtral Equations, **17(7-8)** (2004), 721-736.

[20] Kubo, A. and Suzuki, T., *Mathematical models of tumour angiogenesis*, Journal of Computational and Applied Mathematics, **204(1)** (2007), 48-55.

[21] Kubo, A., Suzuki, T. and Hoshino, H., *Asymptotic behaviour of the solution to a parabolic ODE system*, Mathematical Sciences and Applications, **22** (2005), 121-135.

[22] Kubo, A., Saito, N., Suzuki, T. and Hoshino, H., *Mathematical models of tumour angiogenesis and simulations*, Theory of Bio-Mathematics and Its Applications, in RIMS Kokyuroku, **1499** (2006), 135-146.

[23] Lambert, M., Choquet, D., and Mège, R., *Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the action cytoskeleton* The Journal of Cell Biology, **157(3)** (2002), 469-479.

[24] Levine, H. A. and Sleeman, B. D., *A system of reaction and diffusion equations arising in the theory of reinforced random walks*, SIAM Journal on Applied Mathematics, **57(3)** (1997), 683-730.

[25] Othmer, H. G. and Stevens, A., *Aggregation, blowup, and collapse: The ABC's of taxis in reinforced random walks*, SIAM Journal on Applied Mathematics, **57(4)** (1997), 1044-1081.

[26] Preusser, M., Ribaupierre, S. de., Wöhrer, A., Erridge, S. C., Hegi, M., Weller, M. and Stupp, R., *Current concepts and management of glioblastoma*, Annals of Neurology, **70(1)** (2011), 9-21.

[27] Plank, M. J., Sleeman, B. D. and Jones, P. F., *A Mathematical Model of an In Vitro Experiment to Investigate Endothelial Cell Migration*, Journal of Theoretical Medicine, **4(4)** (2002), 251-270.

[28] Stein, A. M., *Mathematical models for glioma spheroid invasion in 3d collagen-I gel*, A dissertation for the degree of PHD, (2007).

[29] Swanson, K. R., Bridge, C., Murray, J. D. and Ellsworth, Jr., A. C., *Virtual and real brain tumors: using mathematical modeling to quantify glioma growth and invasion*, Journal of the Neurological Sciences, **216(1)** (2003), 1-10.

[30] Stein, A. M., Demuth, T., Mobley, D., Berens, M. and Sander, L. M., *A Mathematical Model of Glioblastoma Tumor Spheroid Invasion in a Three-Dimensional In Vitro Experiment*, Biophysical Journal, **92(1)** (2007), 356-365.

[31] Stokes, C.L., Lauffenburger, D. A., and Williams, S. K., *Migration of individual microvessel endothelial cells: stochastic model and parameter measurement*, Journal of Cell Science, **99(2)**, (1991), 419-430.

[32] Stein, A. M., Vader, D. A., Deisboeck, T. S., Chiocca, E. A., Sander, L. M. and Weitz, D. A., *Directionality of glioblastoma invasion in a 3d in vitro experiment*, arXiv preprint q-bio/0610031, (2008).

[33] Sleeman, B. D. and Wallis, I. P., *Tumour induced angiogenesis as a reinforced random walk: modeling capillary network formation without endothelial cell proliferation*, Mathematical and Computer Modeling, **36(3)**, (2002), 339-358.

[34] Tektonidis, M., Hatzikirou, H., Chauvière, A., Simon, M., Schaller, K. and Deutsch, A., *Identification of intrinsic in vitro cellular mechanisms for glioma invasion*, Journal of Theoretical Biology, *287* (2011), 131-147.

[35] Turner, S. and Sherratt, J. A., *Intercellular adhesion and cancer invasion: a discrete simulation using the extended Potts model*, Journal of Theoretical Biology, **216(1)** (2002), 85-100.