Mathematical models and simulations of glioblastoma invasion

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***Abstract*—**Stein and coworkers have in vitro experiments of U87MG glioblastoma invasion 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 and propose a continuum mathematical model describing the behaviour of invasive cells observed in their experiments. However in their experiments it is seen that the U87MG invasive cells often exhibit more complicated and irregular behaviour than their simulations. We propose a mathematical model, which generalises the radially biased motility term of their model, based on some kind of taxis govering the behaviour of U87MG cells in the experiment. We show a mathematical analysis of our model and give more realistic computer simulations of the behaviour of invasive cells by using our mathematical model.

***Keywords*—**Glioblastoma, tumour, radially biased motility, collagen gel, mathematical model, mathematical analysis, computer simulation, existence of solution, N-cadherin.

I. Introduction

By the remarkable progress in medical technology and image diagnostic technique, in clinical medicine the extent of tumour invasion can be detected precisely. Especially glioblastoma multiforme (GBM) is the most malignant form of brain cancer ([7], [26]). Nevertheless the outcome for patients with glioblastoma is still extremely poor. It confounds the clinical management of glioblastomas due to the high local invasiveness of these cancer cells enabling tumour cells to disperse from the main tumour mass into the surrounding normal brain, so that dispersed glioma cells are out of reach of surgery, radiation, and chemotherapy. On the other hand the details of microscopic behaviour of invasive cells is not still well known and the mechanisms governing cellular level invasion are poorly understood. Actually several mechanisms such as chemokinesis (undirected motility), chemotaxis (directed motility along chemical gradients), haptotaxis, cell-cell adhesion, and cell-cell signaling, upregulation of pro-survival pathways, and microenvironmental cues are seemed to be involved in the process from stationary to a migratory/invasive phenotype ([7], [11]- [13]). Hence the investigation of such cellular level mechanisms could made a valuable contribution to the clinical treatment.

In 2007 Stein et al. [30] presented results from their in vitro 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 (1.1) (see [29]), that allows us to quantitatively interpret the data. Their mathematical model describes a characteristic behaviour of the U87MG invasive cells that they have a strong radial directional motility away from the spheroid center(see Fig. 2(a)). 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 diffuse along a radial 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 cell behaviour we improve their radially biased motility term and give our mathematical model.

The goal of this paper is to gain a better understanding of the mechanism governing invasive cell behaviour. For this purpose we propose a mathematical model and show rigorous mathematical analysis of our model and computer simulations of cell motility closer to real trajectories from the experiment than Stein's simulation in [30], based on our mathematical model. Finally by applying the same manner we show time depending computer simulations of the experiments ([30], [8]).

A. 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:

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

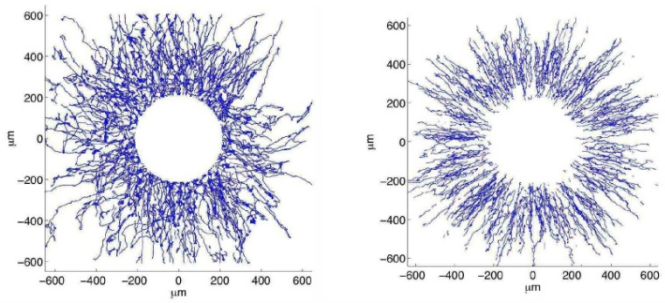
where cell concentration u describes motility along undirected, random paths as a function of position and time, cells throughout the tumour are assumed to proliferate at a constant rate g until they reach a limiting density, $u_{\max}$, the constant D is the diffusion (undirected motion); the larger 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 in their model 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 such biased motility might be due to some attractant in the environment, specified in *Remark1*, 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.

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

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. [30], [30],[32]), invasive cells with the radially directed motility away from the spheroid center make a progress and then they often exhibit more complicated behaviour (see Fig. 1(a), 2(a)). It seems that such behaviour of invasive cells can not be well reproduced by their simulation shown in Fig. 1(b), because their radially biased motility term of (1.2) is of the *linear* form. In order to describe *nonlinear* paths of cells we need to consider a mathematical model generalised the radially biased term in (1.2) to a *nonlinear* term taking account of a mechanism of taxis govering cell behaviour. Then we give rigorous mathematical analysis of our model and computer simulation of cell motility based on it.



(a) (b)

Fig.1 (a) Cell trajectories (b) Simulation of cell trajectories, from in vitro experiment of U87MG glioma tumour 3D invasion in collagen-I gel performed by Stein and coworkers in [32] (cf. [30]).

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

B. Mathematical model generalising the radially biased motility term

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

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

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

$$\begin{aligned}\nabla_r \cdot u &= (r_1,\cdots,r_n)\cdot\left(u_{x_1},\cdots,u_{x_n}\right)\\ &= \nabla u\cdot(r_1,\cdots,r_n) = \nabla\cdot u(r_1,\cdots,r_n)\\ &= \nabla\cdot u\nabla\left(x_1r_1+\cdots+\mathrm{x}_n r_n\right)\end{aligned}$$

replacing $\left(x_1r_1+\cdots+\mathrm{x}_n r_n\right)$ by $\log(\alpha+w)$ for a new unknown function w and a non-negative constant α

$$= \nabla\cdot\left(u\nabla\log(\alpha+w)\right). \tag{1.4}$$

Therefore (1.3) is extended to the following equation.

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

In fact, when we put $w = e^{x_1r_1+\cdots+x_nr_n}-\alpha$, it is seen that $\log(\alpha+w) = x_1r_1+\cdots+x_nr_n$, which indicates that (1.5) is a generalisation of (1.3). (1.5) is considered by Othmer-Stevens [25] in a more general form and can be 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 random walk of the invasive cell along the radial direction away from the center of the spheroid. The following system for (1.5) is applied to a understanding of tumour angiogenesis ([2], [24]).

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

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 decay of w by the interaction between each endothelial cell and some attractant. In this paper we might consider that such attractant is N-cadherin. Therefore in such sense our mathematical model fathfully describes the biological mechanism of the cell migration in the experiment.

Remark 1. *In fact,it is known that N-cadherin is produced on the surface of the invasive tumour cell due to the interaction with collagen-I and is free to diffuse. Such active N-cadherin induces anchoring to actin cytoskelton. It is allowed to cause actintreadmiring and the generation of traction forces against the neighbourhood. Finally it triggers tumour cells migration (see [23]).*

II. Mathematical analysis

In this section we review known mathematical results related to Othmer and Stevens model and apply them to (1.6). They play an important role to carry out the computer simulation.

A. Known result

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

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

Suppose the following assumption (A).

(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 $m\ge[n/2]+3$

$$\mathrm{c}_r(b-\delta_r)<\chi(s_1+b,s_2)\in C^m(R\times R_+), \quad (2.4)$$

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

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 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

$$\begin{aligned}(h,k)(t)&=\int_\Omega h(x,t)k(x,t)\,dx,\\ \|h\|_l^2(t)&=\sum_{|\beta|\le l}\left\|\partial_x^\beta h(\cdot,t)\right\|_{L^2(\Omega)}^2,\end{aligned} \quad (2.6)$$

where $\partial_x=\left(\partial_{x_1},\cdots,\partial_{x_n}\right), \partial_{x_i}=\dfrac{\partial}{\partial x_i}$, $i=1,\cdots,n$ 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 $0<\lambda_i\le\lambda_2\le\cdots\to+\infty$, 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 $\lambda_1\ne 0$ 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*

$$u(x,t)\left(=a+bt+v(x,t)\right)\ \in\bigcap_{i=0}^{1}C^i\left([0,\infty);H^{m-i}(\Omega)\right)$$

to (NE) *such that for* $\bar{u}_1=|\Omega|^{-1}\int_\Omega u_1(x)\,dx$

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

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 III.*

B. Application to mathematical models

(i) 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

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

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

$$P_t = D\Delta P - D\nabla\cdot P\nabla\log\Phi(W),\ W_t = \pm kWP,$$

$$in \qquad \Omega\times(0,\infty) \qquad (2.8)$$

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

$$P(x,0) = P_0(x), W(x,0) = W_0(x) \ge 0, \qquad in \quad \Omega \qquad (2.10)$$

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

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

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).

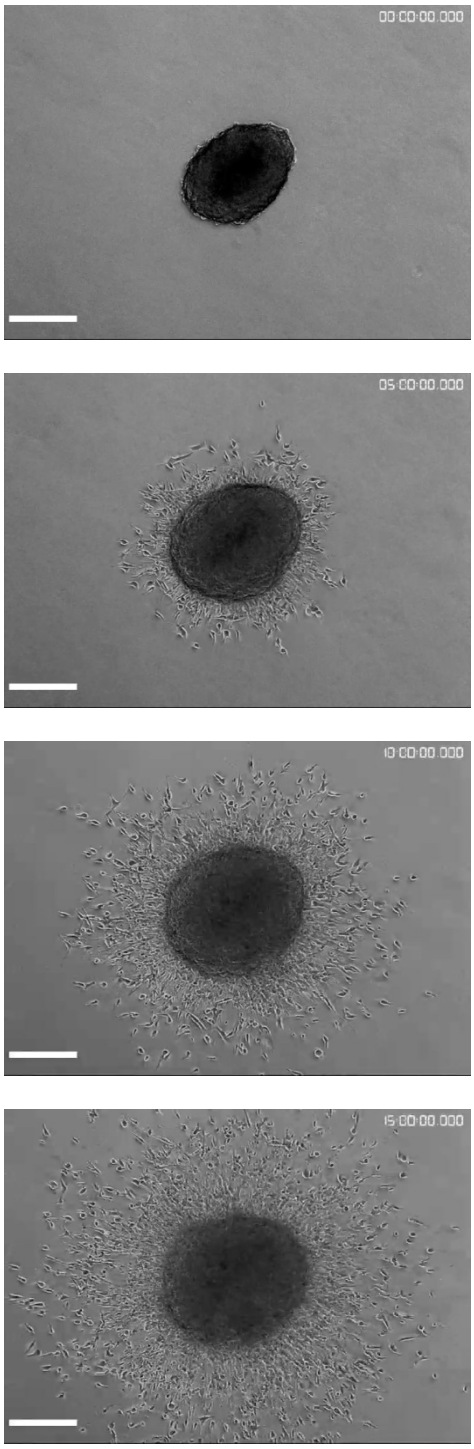
We can carry out computer simulations of these models (i)-(ii) by the rule of reinforced random walk because Othmer-Stevens model is a continuum model of reinforced random walk (see Davis [6]). Since (1.6) is considered as a special case of Othmer-Stevens model, the simulation of (1.6) can be conducted by using the rule of reinforced random walk, which shown in Fig. 3 in the next section. As mentioned in Remark 1, the cause of radially biased motility of invasive cells mainly depends on N-cadhelin, which in our model induce random walk radially biased.

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

III. Computer simulations

In this section we carry out random walk type of computer simulation based on our mathematical model following to Sleeman and Wallis [33] (see [25]).

In Fig. 2(a) we show photos of the 2D projection of the experiment in vitro of U87MG 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]). In the experiment of glioma tumour 3D invasion in collagen gel, invasive cells with the radially directed motility away from the spheroid center make a progress and later they often exhibit complicated behaviour (see Fig. 2(b)).



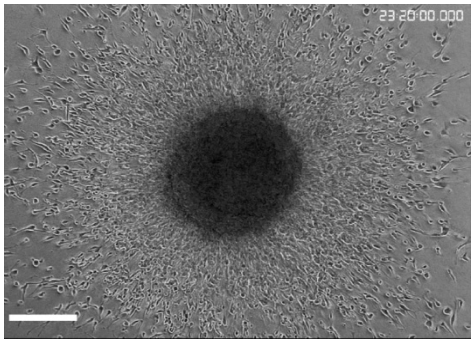


Fig. 2(a) The photos of the 2D projection of the experiment in vitro of glioma tumour 3D invasion in collagen gel performed by Eke and coworkers in [8] for one day. First the tumour spheroid is set at the center of Collagen-I gel. Then by the interaction between Collagen-I and glioma cells cell-cell adhesion of tumour cells is weaken and they gain the ability of migration and invasion. They diffuse in the radial direction away from the spheroid center and make a progress.

In the last image of Fig. 2(a) at t=23:20 we choose seven typical curved paths of invasive cells from the experiment and draw them as solid lines marking by (a)-(c): moderately curved paths, (d)-(g): greatly curved trajectries of invasive cells on the picture (see Fig. 2(b)). In Fig. 3 we show the simulations of the solid lines of Fig. 2(b).

All the computer simulations shown in this section are conducted by Mathematica 8.

We can reproduce (a)-(g) of Fig. 2(b) by using 3D random walk type of simulations based on our mathematical model in Fig. 3. It is obviously much closer to the real paths than Stein's type of simulation Fig. 1 (b).

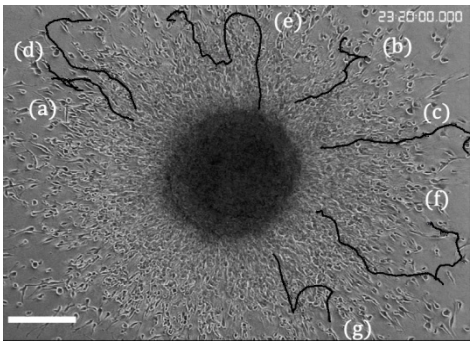


Fig. 2(b) The last image of Fig. 2(a) and trajectories of typical motility of invasive cells (a)-(g). The path (c) shows that the cell first radiates and after that turns around greatly. In the path (b) it is observed that the cell changes the direction several times. In the path (a) once the cell arrives 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 much more greatly.

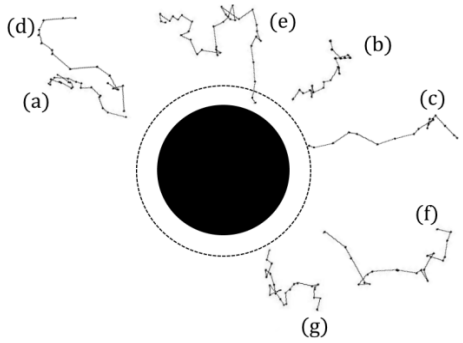
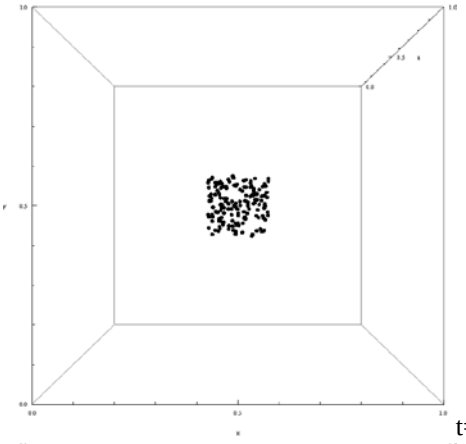
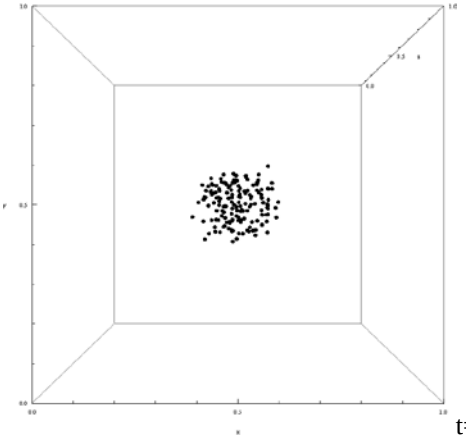


Fig. 3 2D projection of our 3D computer simulations of the experiment by Eke et al.

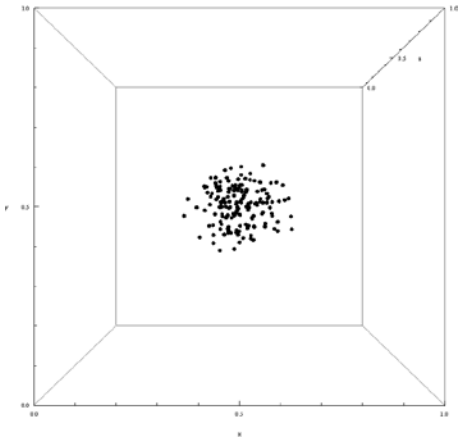
In the following we show 3D simulations of the cell behaviour observed in the the experiment of U87MG glioma 3D invasion by Eke et al., which are based on the same manner as used for (a)-(g) in Fig. 3. First 180 particles is set in the center and the change of the motility of them is observed along the axis of time.

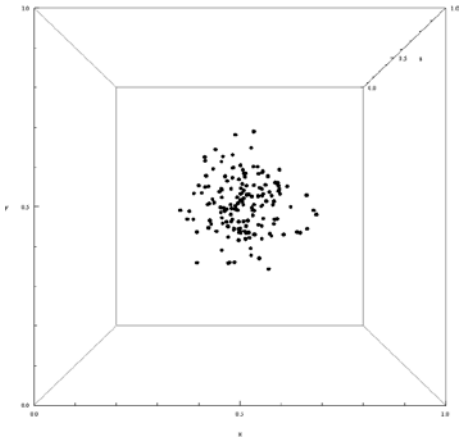


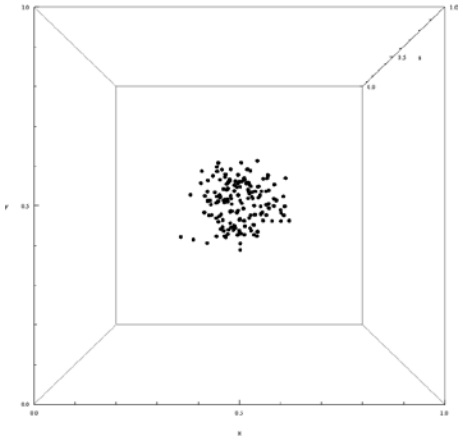
t=0.8h



t=1.6h


t=2.4h


t=5.6h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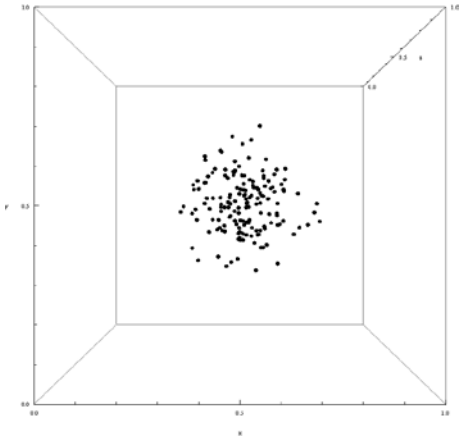
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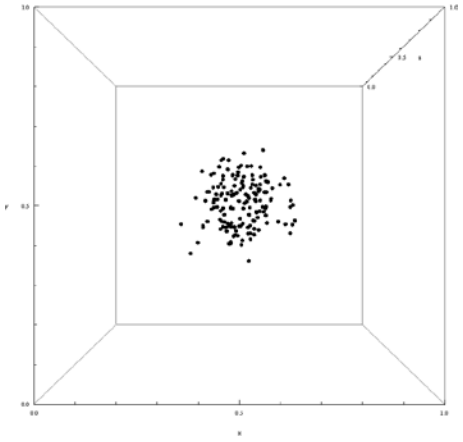
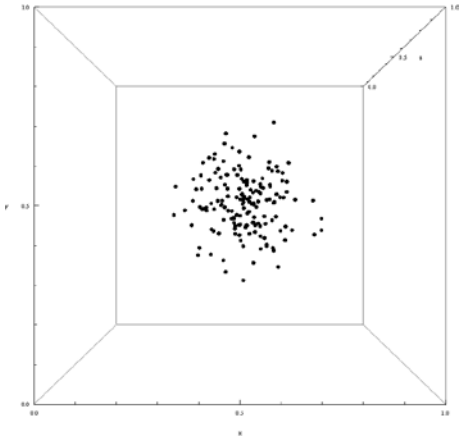
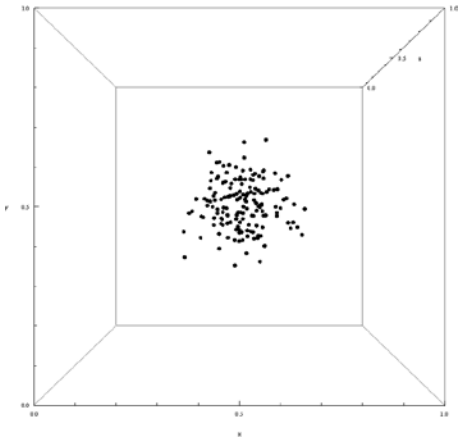

t=4.0h


t=7.2h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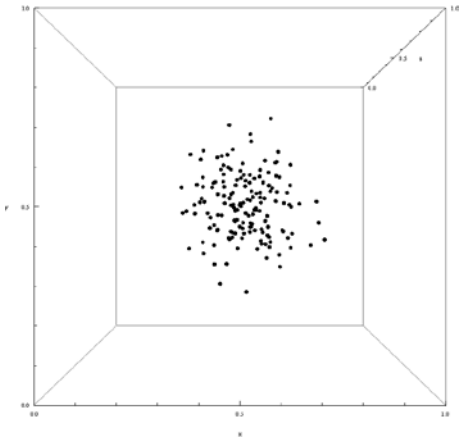
t=8.0h

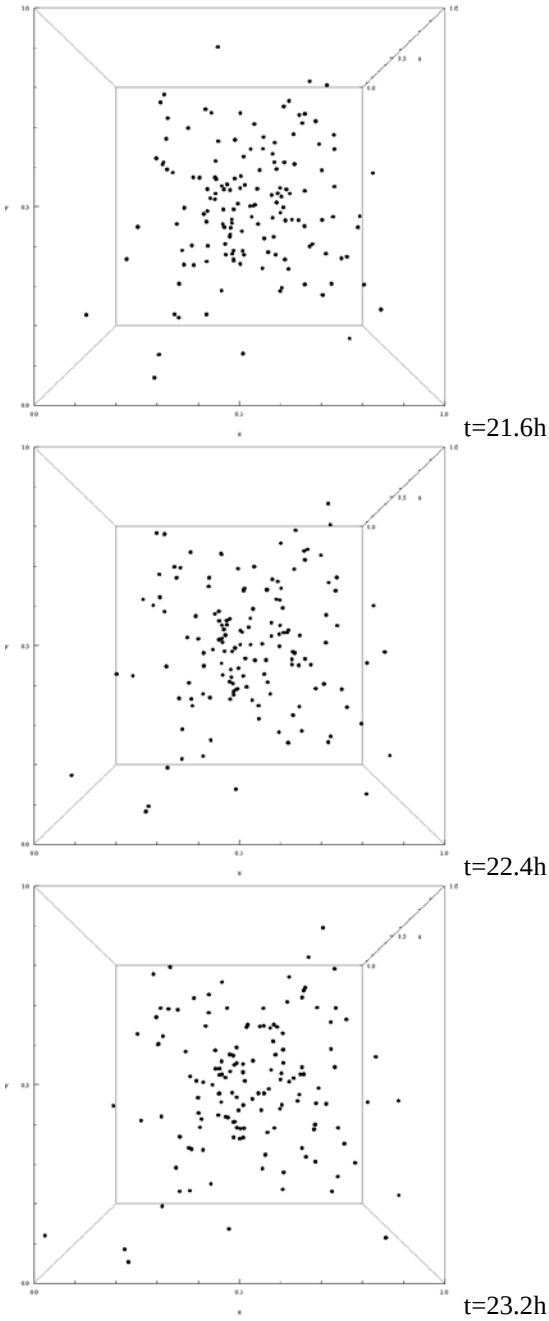

Fig. 4 Simulations of Fig. 2(a) by 180 particles applied the manner used to obtain (a)-(g) in Fig. 2(b). It is observed that the extent of the particles is corresponding to the one of invasive cell population in the experiment along the axis of time.

IV. Conclusion

The data of the in vitro experiment by Stein et al. , Eke et al. imply that the tumour cells start to move radially away from the tumour spheroid and after that the velocity decreases in the radial direction. It seems to be very important to gain a mathematical understanding of the mechanism of invasion in such in vitro experiment. However in the mathematical model given by Stein et al. [30] the radially biased component implies that the cell motility with a constant velocity and a radially linear direction is quite different from real cell paths observed in the experiment.

Considering that the cause of radial bias is due to N-cadhelin, we propose a mathematical model, which improves 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], Stein et al. [30] [32].

In fact, we choose some typical paths of U87MG cells in the experiment as shown in Fig. 2(b). We show a rigorous mathematical analysis of our model and give computer simulations corresponding to solid lines in Fig. 2(b) based on our mathematical model, which realise more realistic simulation of invasive cell than Stein's type of ones.

Finally we give simulations along the axis of time of the experiment by Eke et al., which is same type of Stein et al, based on the same manner used for the simulations (a)-(g) in Fig. 3. We can observe that the extent of the particles is corresponding to the one of invasive cell population in the experiment.

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