A mechanically coupled reaction–diffusion model that incorporates intra-tumoural heterogeneity to predict in vivo glioma growth

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While gliomas have been extensively modelled with a reaction–diffusion (RD) type equation it is most likely an oversimplification. In this study, three mathematical models of glioma growth are developed and systematically investigated to establish a framework for accurate prediction of changes in tumour volume as well as intra-tumoural heterogeneity. Tumour cell movement was described by coupling movement to tissue stress, leading to a mechanically coupled (MC) RD model. Intra-tumour heterogeneity was described by including a voxel-specific carrying capacity (CC) to the RD model. The MC and CC models were also combined in a third model. To evaluate these models, rats (n = 14) with C6 gliomas were imaged with diffusion-weighted magnetic resonance imaging over 10 days to estimate tumour cellularity. Model parameters were estimated from the first three imaging time points and then used to predict tumour growth at the remaining time points which were then directly compared to experimental data. The results in this work demonstrate that mechanical–biological effects are a necessary component of brain tissue tumour modelling efforts. The results are suggestive that a variable tissue carrying capacity is a needed model component to capture tumour heterogeneity. Lastly, the results advocate the need for additional effort towards capturing tumour-to-tissue infiltration.

1. Introduction

A fundamental challenge in the care of cancer patients is the limitation of standard radiographic measurements to accurately evaluate patient response and capture tumour growth kinetics. Biophysical models that incorporate patient-specific measurements may be able to address this challenge by providing accurate predictions of tumour growth and treatment response through which clinical care can be guided [1]. We [2–7] and others [8–12] have proposed that a practical way forward is to initialize and constrain predictive models via imaging data that can be acquired non-invasively, in three dimensions, and at numerous time points. Examples of such data include magnetic resonance imaging (MRI) or positron emission tomography measurements that are routinely available and can characterize tumour vascularity and perfusion [13,14], tumour cellularity [15], metabolism [16] and hypoxia [17]. Towards this effort we are developing biophysical models that are parametrized from a subject’s own imaging measurements and evaluating them in a pre-clinical model of glioma.

A common predictive model in the mathematical analysis of tumours is a reaction–diffusion (RD) model which describes the proliferation (reaction) and movement (diffusion) of tumour cells. Several groups have investigated...
neoplastic growth using variations of this model in several
organ sites (brain [2,8,10,11,18], breast [4,5] and pancreas
[19]). In the pre-clinical setting, we have previously used
MRI data collected at three time points to estimate rat-specific
diffusion and proliferation parameters which were then used
to predict the in vivo spatio-temporal evolution of the tumour.
These predictions were then directly compared to experimental
MRI data collected at future time points. We previously
observed [2] the RD model incompletely described in vivo
C6 glioma growth at both the global (high error in tumour
volume, low agreement in tumour shape) and local (high
error in voxel cell number) levels. In this study, we seek to
modify the standard RD model to improve the accuracy of
predictions at both the global and local levels.

One possibility for the high global level errors is the assump-
tion that the diffusion coefficient is constant in time. In the RD
model, tumour growth is restricted only by the bounds of the
simulation domain (e.g. the skull in the case of a rat glioma)
and not inhibited by the surrounding brain tissue. However,
the force exerted by the surrounding tissue [20] during tumour
expansion is a critical biological interaction that can alter
both tumour expansion [21] and shape [22]. This mechanical–
biological interaction between the tumour mass and surrounding
tissue mass has been previously incorporated into modelling
brain [10,11,18,23], breast [4,5], kidney [24] and pancreatic
[9,19] cancer. Here, we apply it to modelling in vivo C6 glioma
growth using a mechanically coupled (MC) RD model.

Similarly, the high local level errors observed in vivo are a
result of fundamental limitations of the standard RD model
at describing intra-tumoural heterogeneity. More specifically,
with a temporally constant proliferation rate and carrying
capacity the cell number will saturate at steady state within
the tumour resulting in a homogenous distribution of
tumour cells. The limitation of temporally constant proli-
feration can be addressed by incorporating additional equations
or terms into the RD model that result in cell death or altera-
ton of their proliferation status [25,26]. However, parametriz-
ing these models on a subject-specific basis is challenging as they
often have many free parameters that must be assigned in
some reasonable way. Heterogeneity in cell density can also
be obtained through spatially varying the carrying capacity
which may occur due to physical limitations (e.g. decrease in
available space to grow) and environmental limitations
(e.g. poorly perfused, low nutrient concentration). The physical
limitations on growth can be incorporated through the use of
imaging measurements which can provide estimates of the
vascular and extracellular–extravascular volume fractions
[27]. Similarly, models that incorporate vascular growth or
remodelling [28] can also account for these spatial variations
in carrying capacity. In this work, we investigate a simplified
alternative to these approaches to build in heterogeneity by
linking the physical and environmental limitations on carrying
capacity to a single lumped model parameter which can be
estimated throughout the tumour from data observations
(this model is termed the carrying capacity RD model, or CC).

In this contribution, we seek to evaluate the ability of the MC,
CC and MC–CC (the combination of the MC and CC models)
models to predict future tumour growth. Using serial diffusion-
weighted MRI (DW-MRI) data acquired in a murine glioma
model, we first estimate rat-specific model parameters to initialize
and constrain the RD, MC, CC and MC–CC models for predict-
ing the spatio-temporal tumour evolution. Model predictions are
then directly compared to the future MRI measurements.

2. Theory

2.1. Reaction – diffusion model

The RD model, equation (2.1), describes the change in the
distribution and number of tumour cells due to the random
diffusion of tumour cells (diffusion) and proliferation (reaction):

$$\frac{\partial N(\vec{x},t)}{\partial t} = \nabla \cdot \left[ D(\vec{x}) \nabla N(\vec{x},t) + k(\vec{x})N(\vec{x},t) \right] \times \left(1 - \frac{N(\vec{x},t)}{\theta} \right),$$

(2.1)

where $N(\vec{x},t)$ is the number of cells at three-dimensional
position $\vec{x}$ and time $t$, $D(\vec{x})$ is the cell diffusion coefficient at
position $\vec{x}$, $\theta$ is the cellular carrying capacity (i.e. the maximum
number of cells a region can support), $k(\vec{x})$ is the tumour cell
proliferation rate, and $\vec{x}$ indicates the three-dimensional Car-
tesian coordinates $(x, y, z)$. Measurements from DW-MRI are
used to provide estimates of $N(\vec{x},t)$ or $N_{\text{mean}}(\vec{x},t)$ at several
time points [2,29]. These $N_{\text{mean}}(\vec{x},t)$ are then used to solve an
inverse problem of estimating model parameters $P$ for equation
(2.1). For the RD model $P(P_{\text{RD}})$ includes voxel-wise values of
$k(\vec{x})$ and two global $D(\vec{x})$ parameters, one for all voxels
within the white matter ($D_w$) and one for all voxels within the
grey matter ($D_g$). A three dimensions in space ($\Delta x = 250 \mu m$,
$\Delta y = 250 \mu m$, $\Delta z = 1000 \mu m$), fully explicit in time (time
step = 0.01 days) finite difference (FD) simulation is used to
solve the forward problem of equation (2.1) written in Matlab
(Mathworks, Natick, MA, USA). $N(\vec{x},t)$ has no diffusive flux
at the brain tissue boundaries (Neumann boundary condition).

2.2. Mechanically coupled model

To address the high global level errors produced by the RD [2]
model, a MC RD model [4,5,18] will be used. The MC model is
based on observations that the growth of tumour spheroids
is inhibited as the stiffness of the embedded matrix increases [21].
Furthermore, as the tumour volume expands the tumour
induces significant mechanical stress on the surrounding tissue
[30] resulting in a mass effect. Thus, as the tumour expands
there is an increasing amount of mechanical stress which can
restrict further expansion by the tumour. These biophysical
effects are incorporated in the MC model by altering equation
(2.1) to incorporate a diffusion coefficient, equation (2.2), that is
spatially and temporally variant in response to local tissue stress:

$$D(\vec{x},t) = D_0 \cdot e^{-\lambda_1 \sigma_{\text{vm}}(\vec{x},t)},$$

(2.2)

where $D_0$ represents tumour cell diffusion in the absence
of mechanical restrictions, $\lambda_1$ is an empirically derived stress-
tumour cell diffusion coupling constant, and $\sigma_{\text{vm}}(\vec{x},t)$ is the
von Mises stress [4,5,7,18]. A single value of $D_0$ is used to
describe tumour cell diffusion in the absence of mechanical
restrictions. We assume that the mechanical environment (and
the variations in local mechanical properties) is what primarily
drives the local diffusion of tumour cells, and thus the variations
in tumour shape. As the local von Mises stress increases, tumour
cell movement will be inhibited by a decrease in the tumour cell
diffusion coefficient, $D(\vec{x},t) \cdot \sigma_{\text{vm}}(\vec{x},t)$ is determined by calcu-
lating the tissue displacement ($\vec{u}$) caused by the growing tumour.
The following equation, which is the linear elastic isotropic
mechanical equilibrium equation with an expansion force
related to $N(\vec{x},t)$, is used to solve for $\vec{u}$:

$$\nabla \cdot G \nabla \vec{u} + \nabla \frac{G}{1 - 2\nu} (\nabla \cdot \vec{u}) - \lambda_2 \nabla N(\vec{x},t) = 0,$$

(2.3)
where \( G \) is the shear modulus, \( \nu \) is Poisson’s ratio, and \( \lambda_2 \) represents a tumour cell force coupling constant. Equation (2.3) is solved using the FD method. Briefly, equation (2.3) is rearranged into a matrix description of the system shown in the following:

\[
Mu = \lambda_2 \nabla N,
\]

where \( M \) represents the known FD coefficients from equation (2.3), \( u \) is a vector representing the unknown tissue displacement in the \( x \), \( y \), and \( z \)-directions, and \( \nabla N \) is a vector containing the known gradient of \( N \) in the \( x \), \( y \), and \( z \)-directions. \( M \) is a square \( 3N \times 3N \) coefficient matrix where \( N \) is the number of nodes. This is solved using an iterative approach (Matlab’s \texttt{bicgstab} function) using an incomplete LU decomposition of \( M \) as a preconditioner. \( M \) is built once at the beginning of the simulation, while \( \nabla N \) is updated for each iteration. Boundary conditions were prescribed with no tissue displacement in the normal direction of the boundary, while tissue in the tangential directions was free to move (i.e. slip condition).

2.3. Voxel-specific carrying capacity model

Another shortcoming of the RD model is that at steady state it predicts a homogeneous distribution of tumour cells within the tumour compared to the heterogeneous distribution observed in vivo. The in vivo heterogeneous distribution may occur due to several factors including local space limitations (e.g. variations in local vascularity or extracellular swelling) and local viability (e.g. distance to nearby vasculature, amount of available nutrients). One way to incorporate the heterogeneity observed in vivo is to allow the carrying capacity to vary spatially, effectively lumping geometric and metabolic growth constraints into a voxel-wise parameter describing the local carrying capacity. Thus, we amend equation (2.1) to incorporate a voxel-specific carrying capacity [33]:

\[
\frac{\partial N(x,t)}{\partial t} = \nabla \cdot \left[ \theta_{\text{max}} D(x,t) \nabla \left( \frac{N(x,t)}{\theta(x)} \right) \right] + k(x)N(x,t) \times \left( 1 - \left( \frac{N(x,t)}{\theta(x)} \right) \right),
\]

where \( \theta_{\text{max}} \) is the maximum number of cells a voxel can physically support. The voxel-specific carrying capacity, \( \theta(x) \), alters both the movement (preferential movement to areas with a lower packing fraction versus areas of lower cell number) and proliferation (maximum cell number varies throughout the tumour) of tumour cells. The estimated parameter set, \( P \), for the CC (\( \text{P}_{\text{CC}} \)) model includes voxel-wise \( k(x) \), voxel-wise \( \theta(x) \) and two \( D(x) \) values; one for white matter and one for grey matter. \( \text{P}_{\text{CC}} \) is estimated using the same approach used for the RD model, \( k(x) \) and \( \theta(x) \) are spatially, but not temporally, variant. While it is likely that \( k(x) \) and \( \theta(x) \) have some temporal variation as a tumour grows and some error will be incurred as a consequence, in this preliminary realization, we have chosen to assess the fidelity of our approach with the assumption of these parameters being temporally invariant.

2.4. Combined model (MC–CC)

The fourth model amends the RD model to incorporate both a spatially temporally variant \( D(x,t) \) coupled to local tissue stress (MC) and a spatially variant \( \theta(x) \) (CC). The estimated parameter set, \( P \), for the MC–CC (\( \text{P}_{\text{MC–CC}} \)) model includes voxel-wise \( k(x) \), voxel-wise \( \theta(x) \) and one \( D_0 \) value.

3. Material and methods

3.1. In vivo experiments

For all imaging and surgical procedures, rats were anaesthetized with 2% isoflurane in 98% oxygen. Female Wistar rats (\( n = 14 \), 236–268 g) were anaesthetized and inoculated intracranially with C6 glioma cells (\( 1 \times 10^7 \)) via stereotaxic injection. While the C6 line may not recapitulate all the manifestations of human glioma, it was selected for its widespread use in neuro-oncology, pre-clinical evaluation of imaging methods, and mathematical modelling, as well as its repeatable growth characteristics [34]. A jugular catheter was placed in each rat 8 days after the tumour inoculation surgery for injection of an MRI contrast agent. During each MRI procedure, rat body temperature was maintained near 37 °C by a flow of warm air directed over the animal and respiration was monitored using a pneumatic pillow. The first imaging time point occurred 10 days after inoculation surgery. The imaging measurement time points for each rat are reported in table 1. DW-MRI, contrast-enhanced MRI (CE-MRI) and T1 maps were acquired on a 9.4 T horizontal-bore magnet (Agilent, Santa

Figure 1. The panels depict a single iteration of the MC tumour model. The current values of \( D(x,t) \), \( N_{\text{model}}(x,t) \) and \( k(x) \) are used in a finite difference simulation, step 1, of equation (2.1). The updated \( N_{\text{model}}(x,t) \) is then used to calculate \( \nabla N(x,t) \), step 2. Using \( G \), \( v \), \( A_2 \) and \( \nabla N(x,t) \) equation (2.4) is solved for \( u \) (step 3). \( u \) is then used to calculate the normal shears and stresses, step 4, in order to calculate \( \sigma_{\text{vm}}(x,t) \). \( D(x,t) \) is then updated, step 5, using \( \sigma_{\text{vm}}(x,t) \) in equation (2.2). (Online version in colour.)
Clara, CA, USA) using a 38 mm diameter Litz quadrature coil (Doty Scientific, Columbia, SC, USA). All MR images were acquired over a 32 × 32 × 16 mm³ field of view sampled with a 128 × 128 × 16 matrix. From the beginning of the second through the final imaging session, a mutual information based rigid registration algorithm was used to register the current imaging time point to the initial imaging session.

DW-MRI was acquired using a pulsed fast spin echo diffusion sequence with three orthogonal diffusion encoding directions with b-values of 150, 500 and 1100 s/mm², and Δ/δ = 25/2 ms. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35]. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35]. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35]. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35]. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35]. The apparent diffusion coefficient (ADC) was estimated on a voxel basis using standard methods [35].

The parameter estimation method is shown in figure 2. ADC maps obtained from DW-MRI are used to provide estimates of $N(x, t)$ at several time points (step 1). The optimal parameters were determined by minimizing the sum squared difference between the measured and model estimated tumour cell (i.e. the objective function) using the Levenberg–Marquardt method (steps 2–4). The optimization process terminated when one of the following occurs: error in the objective function stagnated (less than 0.5% change in value between successive iterations), iteration count reaches 1000, or change in parameter values stagnated (less than 0.5% change in value between successive iterations). A $3 \times 3$ voxel Gaussian filter was applied to $N_{\text{meas}}(x, t)$ prior to the start of the parameter optimization to reduce the effects of noise within individual voxels. The model parameters were constrained from 0 to 10 day⁻¹ for $k(x)$, $0.35 \times \theta_{\text{max}}(x)$ to $\theta_{\text{max}}(x)$ for $\theta$, and 5000–500 000 μm² day⁻¹ for $D_w$, $D_p$ and $D_0$. The estimated model parameters were within these constraints and did not optimize to the lower or upper constraints. Parameter constraints were selected based on previous experience [2] and biologically relevant ranges observed in the literature. The upper constraints for the diffusion coefficients were used in a Von Neumann stability analysis [40] to determine the maximum time step for FD simulations to remain monotonic and stable. A single time step of 0.01 day was used for all simulations. $k(x)$ was estimated voxel-wise within the tumour ROI and assigned 0 elsewhere. Similarly, for the CC and MC–CC models, $\theta(x)$ was estimated voxel-wise within the tumour ROI and assigned the maximum value elsewhere. Single values for $D_0$, $D_p$ and $D_w$ were estimated for the whole brain.

An in silico study was performed for each model to evaluate the robustness of the parameter optimization approach to measurement noise. Briefly, for each model 250 noisy datasets were generated by adding random noise from a normal distribution with a standard deviation based on the repeatability of ADC measurements [35] (as described in [2]). Model parameters were then estimated from the noisy datasets and then compared with the true model parameters used to grow the synthetic datasets. Model parameter optimization used the Lonestar 5 Cluster at the Texas Advanced Computing Center (TACC) of the University of Texas at Austin.

### 3.3. Forward evaluation and error analysis

After optimization of $P$ for each model, these values were then used in an FD simulation initialized at day 10 and ‘grown’ to each rat’s final imaging measurement (figure 2, step 5). The simulated tumour growth ($N_/\text{FD}$, $N_/\text{MC}$, $N_/\text{CC}$, $N_/\text{MC–CC}$) was then sampled at time points corresponding to the experimentally acquired imaging time points. As the tumour expanded into regions where an estimate of $k(x)$ was not obtained, $k(x)$ was assigned using a local average of available non-zero $k$’s within a $3 \times 3 \times 3$ voxel kernel. This approach results in a smooth occuring. A discussion section.
Table 2. Synthetic dataset optimization results. Per cent absolute error in model parameter means (95% CI).

<table>
<thead>
<tr>
<th>model parameters</th>
<th>RD</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_u)</td>
<td>2.39 (0.18)</td>
<td>2.19 (0.20)</td>
</tr>
<tr>
<td>(D_g)</td>
<td>2.20 (0.16)</td>
<td>2.26 (0.21)</td>
</tr>
<tr>
<td>(k(x))</td>
<td>5.55 (0.60)</td>
<td>5.46 (0.62)</td>
</tr>
<tr>
<td>(\theta(x))</td>
<td>n.a.</td>
<td>1.39 (0.13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>model parameters</th>
<th>MC</th>
<th>MC–CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_0)</td>
<td>0.74 (0.06)</td>
<td>3.36 (0.23)</td>
</tr>
<tr>
<td>(k(x))</td>
<td>1.48 (0.20)</td>
<td>6.92 (0.67)</td>
</tr>
<tr>
<td>(\theta(x))</td>
<td>n.a.</td>
<td>3.44 (0.29)</td>
</tr>
</tbody>
</table>

proliferation map outside of the estimated region with areas of predominately high or low proliferation rates moving outwards as the tumour grows.

Error between predicted and the measured tumour growth (identified using CE-MRI data) was assessed at the global and local levels. At the global level, error was assessed by calculating the per cent error in tumour volume and the average surface distance (ASD). The ASD is the average minimum distance between a voxel on the surface of the model predicted tumour volume and a voxel on the surface of the measured tumour volume. At the local level, error was assessed by calculating the average per cent error in cell number, the Pearson correlation coefficient (PCC), and the concordance correlation coefficient (CCC). Per cent error in tumour volume was used to assess the error in volume estimates, while the agreement between ROI shapes was assessed using the ASD. The per cent error in tumour volume was computed for each model by comparing the predicted tumour volume to the measured tumour volume on days 15–20. Similarly, the predicted tumour ROIs on days 15–20 were compared to the measured tumour volume on the same days to compute the ASD. The error at the local level was assessed at days 15–20 by calculating at each voxel the per cent error between \(N_{\text{model}}(x, t)\) and \(N_{\text{meas}}(x, t)\). The voxel-wise error is then averaged to compute the average per cent error in cell number. The PCC and CCC were also calculated to evaluate the correlation and agreement between voxel-wise cell number and the observed cell number. All results are presented as the mean and 95% CI when appropriate.

A one-way analysis of variance (ANOVA) was used to evaluate the differences in global and local errors between the RD, MC, CC and MC–CC models. Tukey’s honest significant difference test was then used for pairwise comparisons. A p-value less than 0.05 was considered significant. Models that had significant differences at all 4 time points were considered to be highly suggestive of a fundamental model requirement, while models with significant differences at 3 time points were considered moderately suggestive. No conclusions were made for models with significant differences at 2 or fewer time points.

4. Results

4.1. Parameter optimization

Table 2 shows the results which evaluate the optimization process for synthetic datasets for the RD, CC, MC and MC–CC models. The absolute value of error in estimated parameter values ranged from 0.74% to 6.92% for all models and model parameters.

4.2. Mechanically coupled model

Figure 3 shows the comparison at the global level of the RD and MC model predictions for rat 7. The MC model resulted in lower error in tumour volume relative to the RD model at day 15. Increased error is observed for the RD compared to the MC model at the final imaging time point (27.48% for the RD and 7.89% for the MC model). (Note: for clarity, the CC model is excluded from this figure as its contours overlap with the RD model.) Figure 3d shows a steady increase in per cent error in tumour volume for the RD model while the MC model remains below 8%. Similarly, the MC model results in a decreased ASD (0.22 mm for the MC and 0.33 mm for the RD), which is most noticeable at the later time points.

4.3. Voxel-specific carrying capacity model

Figure 4 shows the local error analysis for the CC model from the central tumour slice of rats 3, 7 and 10 on day 16. ND\(\text{RD}(x, t)\), figure 4b, results in a more uniform distribution of tumour cells in comparison to what is observed in vivo, figure 4a. Elevated errors are observed in areas corresponding to low \(N_{\text{meas}}(x, t)\) for rats 3, 7 and 10 (13.27 ± 0.87%, 8.46 ± 0.70% and 23.10 ± 1.17%, respectively). Lower cellularity regions in \(N_{\text{meas}}(x, t)\) are reflected in the fitted carrying capacity, figure 4d. The predictions of the CC model, figure 4c, resulted in less uniform distributions of tumour cells compared to ND\(\text{RD}\). In particular, rat 3 captures some of the low cellularity regions observed in \(N_{\text{meas}}(x, t)\). Rats 3, 7 and 10 showed reduced error (12.12 ± 0.90%, 5.08 ± 0.68% and 6.27 ± 0.51%, respectively) for the CC model, figure 4e, compared to the RD model, figure 4c. (Note: for clarity, the MC model is not included in this figure as it does not improve the local level errors and results in similar error maps as the RD model.)

Figure 5 compares the RD and CC models, along with \(N_{\text{meas}}(x, t)\) at the local level. Rat 3 shows significant differences (p < 0.05) between the RD and CC model (for \(N_{\text{meas}}(x, t)/\theta < 0.95\)) with the RD model resulting in a mean increase in error of 7.87% over the CC model. Rat 7 shows significant differences (p < 0.05) observed between the RD and MC models for rat 10, where the RD model also resulted in a mean increase of 11.28% error compared with the CC model, figure 4c. Rat 3 shows significant differences (p < 0.05) compared to what is observed in vivo, figure 4a. Elevated errors are observed in areas corresponding to low \(N_{\text{meas}}(x, t)\) for rats 3, 7 and 10 (13.27 ± 0.87%, 8.46 ± 0.70% and 23.10 ± 1.17%, respectively). Lower cellularity regions in \(N_{\text{meas}}(x, t)\) are reflected in the fitted carrying capacity, figure 4d. The predictions of the CC model, figure 4c, resulted in less uniform distributions of tumour cells compared to ND\(\text{RD}\). In particular, rat 3 captures some of the low cellularity regions observed in \(N_{\text{meas}}(x, t)\). Rats 3, 7 and 10 showed reduced error (12.12 ± 0.90%, 5.08 ± 0.68% and 6.27 ± 0.51%, respectively) for the CC model, figure 4e, compared to the RD model, figure 4c. (Note: for clarity, the MC model is not included in this figure as it does not improve the local level errors and results in similar error maps as the RD model.)

Figure 6 shows the global level of the RD and MC model predictions for rat 7. The RD model resulted in lower error in tumour volume relative to the RD model at day 15. Increased error is observed for the RD compared to the MC model at the final imaging time point (27.48% for the RD and 7.89% for the MC model). (Note: for clarity, the CC model is excluded from this figure as its contours overlap with the RD model.) Figure 3d shows a steady increase in per cent error in tumour volume for the RD model while the MC model remains below 8%. Similarly, the MC model results in a decreased ASD (0.22 mm for the MC and 0.33 mm for the RD), which is most noticeable at the later time points.

4.4. Combined model (MC–CC)

Figures 6–8 show the results of the global and local error analysis for the MC–CC model. Figure 6 shows at day 20, the RD and MC models (figure 6c,g, respectively) both fail to capture the low cell-density regions seen within \(N_{\text{meas}}(x, t)\) resulting in high local level error (15.95 ± 0.89% and 17.01 ± 1.14%, respectively). Conversely, the CC and MC–CC
models (figure 6e, i, respectively) capture some low cellularity regions in $N_{\text{ext}}(x, t)$ resulting in decreased local level errors ($7.96 \pm 0.79\%$ and $9.05 \pm 0.96\%$, respectively). Increased error in cell number is observed in the centre of the tumour (greater than 40%) compared to the periphery (less than 10%). All four models have low global level errors at day 15 (less than 16%). At days 18 and 20, however, the RD and CC models overestimate tumour growth (greater than 41%) compared to the MC and MC–CC models (less than 6%).

Visually, it is evident that the MC–CC model combines the benefits of both the MC (low global level errors) and CC (low local level errors) models in predicting future tumour growth.

Figure 7a–c shows global and local errors applied to the complete tumour volume for rat 1, while figure 7d–f shows the analogous results for the cohort. Less than 6% error in tumour volume, figure 7a, was observed for the MC models (MC and MC–CC) at all time points, whereas the non-MC models (RD and CC) had errors in tumour volume ranging from 13.42% to 84.12%. All models had low average surface distances less than 0.31 mm, figure 7b, at days 15 and 16.

Conversely, on day 20 the RD and CC models had ASDs greater than 0.89 mm compared with the MC and MC–CC models which had ASDs less than 0.40 mm. The RD and MC models both had increased error ranging from 16.13 to 30.12% in voxel cell number, figure 7c, compared to the CC and MC–CC models which ranged from 9.55 to 17.75%.

Similar results are seen in the cohort (figure 7d–f). The non-MC models resulted in increasing volume error, figure 7d, over time ranging from 16.08 to 50.37%, whereas the MC models had less than 8% error in tumour volume estimates. Similarly, figure 7e shows that the MC and MC–CC models both have decreased average surface distances ranging from 0.15 to 0.33 mm compared with the RD and CC models which ranged from 0.19 to 0.60 mm. Models with a voxel-specific carrying capacity (CC and MC–CC models) reduced local level error, figure 7f, from greater than 19.92% error (RD and MC models) to less than 15.04% error (CC and MC–CC models).

The results of the pairwise testing following statistically significant ANOVA results ($p < 0.05$) indicated that the MC...
and MC–CC models had significantly reduced error in tumour volume at all time points compared to the RD model. Similarly, the MC and MC–CC models had significantly reduced error in tumour volume at days 16, 18 and 20 compared with the CC model. For local level error (error in voxel cell number), the CC and MC–CC models had significantly reduced error at all time points compared to the RD model. Additionally, the MC–CC model had reduced local error compared with the MC model at days 15, 16 and 20. No significant differences (p-values for F-test ranged from 0.053 to 0.193) were observed between model values of ASD.

Figure 8 shows the PCC and CCC results for the RD and CC–MC models. Figure 8c shows a reduction in correlation and agreement between \( N_{\text{RD}}(x,t) \) and \( N_{\text{meas}}(x,t) \) from day 15 (PCC = 0.87, CCC = 0.70) to 20 (PCC = 0.68, CCC = 0.46). Figure 8d also shows decreased correlation and agreement between \( N_{\text{MC–CC}}(x,t) \) and \( N_{\text{meas}}(x,t) \) from day 15 (PCC = 0.92, CCC = 0.87) to 20 (PCC = 0.86, CCC = 0.72). The MC–CC model resulted in an increased level of agreement and correlation at all time points compared to the RD model.

![Figure 8](image)

### 5. Discussion

The results of the model analysis showed that global error can be significantly improved by coupling local tissue stress to tumour cell diffusion and that local error can be improved by fitting for a voxel-specific carrying capacity. Global error analysis showed that the MC models (MC, MC–CC) were significantly more accurate in describing tumour volume than the RD and CC models. Generally, global level error for the MC models (MC, MC–CC) remained relatively constant from days 15 to 20 compared to the non-MC models (RD, CC) which grew from days 15 to 20. This suggests that the MC model could potentially provide better global level predictions past day 20 compared to the non-MC models. The local level analysis showed that incorporating a voxel-specific carrying capacity (CC, MC–CC) reduced local level error compared with when a global value was used (RD, MC). The MC–CC model benefits from both a reduced global level error (lower error in tumour volume, lower ASD) provided by the MC component of the model and reduced local level error (less error in local cell number) provided by the CC component of the model. Additionally, a strong level of agreement and correlation, compared to the RD model, was observed between the MC–CC model and measured cell number at the voxel level. This work demonstrates that individualized model predictions can be made accurately using parameters estimated from a subject’s own imaging measurements.

To evaluate the model-prediction framework we used a pre-clinical model of glioma (the C6 line) injected orthotopically...
Mechanical forces play a critical role in the growth of tumours [20,21] and the interaction with these forces needs to be considered in the development of accurate mathematical models. Cancer development results in complex changes to the mechanical microenvironment between a tumour and its supporting stroma and is likely to affect the growth and invasion of tumour growth [41–43]. We have focused on the inhibitory role of local mechanical stress on gross tumour growth [18]. Without mechanical coupling, error in predicted tumour volume worsens over time (increasing approx. three-fold from days 15 to 20). The results of this model analysis, suggested that incorporating an MC tumour cell diffusion coefficient is critical for accurately predicting gross tumour growth.

It results in significant decreases in global level error (per cent error in tumour volume) when compared to the RD model. Furthermore, the gross inhibitory effect of local tissue stress observed modelled here accurately describes in vivo C6 glioma growth. One of the limitations of this current study is that the shear modulus was uniquely assigned to only a few identifiable regions based on literature values. While this current approach still results in reduced global level errors due to inhibitory effect of the MD model [21], a more tissue-specific approach could potentially more accurately describe glioma morphology [44]. More accurate assignment of these tissue-specific properties (via, for example, magnetic resonance elastography after tumour implantation [45]) may improve the predictions of future tumour morphology. These results suggest the importance of mechanical forces on accurate tumour growth predictions. Other efforts have included the influence of mechanical forces on prediction of breast [5], pancreatic [46] and kidney [24] cancers. This model could be adapted for other cell lines or human cancers using a different degree of coupling by changing $\lambda_1$.

Mathematically modelling cell interactions at the local level is a large field [28,47–50] with complexity ranging from a single equation to models with several coupled equations. However, parametrizing these models in a subject/tumour-specific approach often would require highly invasive measurements to accurately capture the in vivo behaviour. In this work, a simple alteration of the RD model to include a voxel-specific carrying capacity resulted in reduced local level errors. For the RD and MC models, $\theta$ is based on voxel dimension and average tumour cell size. In the CC model, the voxel-specific $\theta(x)$ is, in essence, a snapshot of the cumulative effects of both physical restrictions and environmental limitations.

The analysis of the CC model suggests that it is significantly better at describing in vivo C6 glioma behaviour. The CC model allows for changes in existing low cell-density regions to be more accurately described (figure 4). Furthermore, the CC model is significantly more accurate in capturing the cell number distribution (figure 5e) observed in vivo. The CC model does, however, fail to capture expanding low cellularity regions as seen in figure 6 which is one of the main limitations to this model. Since $\theta(x)$ is temporally constant, which if it is truly a function of physical and environmental limitations may not be accurate, a higher error may be observed in future predictions if necrotic regions develop. The CC model provides improved local level error for voxels that are saturated in the RD model. $\theta(x)$ calibrates the model to adjust the differences between the calculated physical carrying capacity and the observed cell density. In future work, we plan to investigate the sensitivity of our model parameters to real change in cell density versus experimental noise. At this time, we do not know what degree of change in, for example, $\theta(x)$ represents a significant change over the measurement noise. However, in voxels where $N_{\text{max}}(x,t)$ is decreasing over time (potentially due to necrosis) we would have worsening error as $N_{\text{max}}(x,t)$ continues to decrease. One potential solution is to develop a more complicated and explicit relationship between $\theta$ and physical limitations (vasculature space [27,28], edema [51], non-tumour cells [52] and environmental limitations (availability of glucose and oxygen [47], acidity [52]) that could be evolved temporally as these conditions change. However, it is important to note that such modifications would likely require additional model assumptions and parameters that need additional data sources to define. A second limitation of the
CC model is that it requires two sets of voxel-specific parameters ($k(x)$ and $\theta(x)$) and two global parameters ($D$ for white and grey matter) which may be challenging to accurately estimate in a data limited situation. Model selection criteria [53,54] that balance the goodness of fit and model complexity should be used to determine whether a more complex model is preferred for specific disease settings. Estimating a global carrying capacity may also provide some of the benefit of the locally estimated carrying capacity to account for variations in carrying capacity between disease settings. A spatial regularization process to enforce local continuity (as nearby voxels are likely to influence each other) may improve the convergence and result in a more robust optimization. However, we elected to use a data-driven approach to estimating model parameters rather than constraining our approach with an unknown correlation that is as of yet arbitrarily assigned.

The combined model, MC–CC, provides the best description of the in vivo C6 glioma growth by reducing both global and local level errors. The MC–CC model also shows a very high level of agreement and correlation to the in vivo observed cell number. Furthermore, statistically significant differences between the RD and MC–CC models were observed for both the global and local level metrics. This model is not intended (as-is) to be applied directly to clinical glioma cases which would require additional modification of the model system to account for patient-specific therapeutic interventions. However, our approach could be used as an accurate individualized control against which novel treatments or treatment schedules could be evaluated. In the pre-clinical setting, this model could be used as the foundation for the inclusion of the effects of radiation or chemotherapy that could be used to investigate novel approaches to delivering these therapies. Depending on the quantity of interest, the MC model may be sufficient to provide estimates of tumour volume and location without adding an additional spatially varying model parameter.

There are, however, some key underlying limitations for all of the modelling approaches discussed in this work. First, we use the measured ADC to estimate cellularity at the voxel level. While several studies have shown a strong correlation between histological estimates of cellularity in human brain tumours [55] ($r = 0.77$, $p = 0.007$), breast cancer [56] ($r = 0.54$, $p < 0.01$), extracranial lesions [57] ($r = 0.73$, $p < 0.001$), small animal models of breast cancer [37] ($r > 0.57$, $p = 0.03$), and in vitro studies [36], there are other factors (cell membrane permeability [58], cell size, tortuosity [59], oedema [60] and necrosis [60]) which can also affect the measured

Figure 7. Global and local error results for all models. Per cent error in tumour volume, panels (a,d), the average surface distance, panels (b,e), and the per cent error in voxel cell number, panels (c,f), are shown for rat 1, panels (a–c), and all rats, panels (d–f). For rat 1, increased global level error, panels (a,b), is observed for the RD and CC models compared to the MC and MC–CC models. Similarly, increased local error is observed, panel (c), for the RD and MC models compared to the CC and MC–CC models. Similar results were observed for the cohort. The mean and 95% CI are shown in panels (d–f). Increased error was observed in tumour volume, panel (d), for the RD and CC models (greater than 16%) compared to the MC and MC–CC models (less than 8%). Elevated average surface distances, panel (e), were also observed for the RD and CC models. Less than 16% error was observed at the local level, panel (f), for the CC and MC–CC models. (Online version in colour.)
ADC. Thus, this transformation (i.e. equation (3.1)) from ADC to cellularity is a first-order approximation to the true tumour cellularity. A second limitation of this work is the quantity of imaging measurements that are needed to estimate model parameters. In data limited settings (e.g. standard-of-care clinical studies), our approach will not be tenable. A third limitation of note is that our current model does not provide a means to explicitly alter \(k(x)\) and \(\theta(x)\) as a function of time. It is important for future work to explicitly alter \(k(x)\) and \(\theta(x)\) as a function of time; however, developing a system of equations that can accurately capture this dynamic process will be challenging. We are currently working to address this fundamental shortcoming of the model.

6. Conclusion

The standard RD model poorly predicts the spatio-temporal evolution of in vivo C6 glioma growth in rats resulting in high global and local level errors. Comparing across figures 6c–i and 7, it is clear that mechanically coupling local tissue stress to tumour cell movement is a fundamental requirement towards accurate tumour growth models. While more subtle, figure 8 demonstrates that a voxel-specific carrying capacity is likely a necessary ingredient for improving local predictions of the cellular heterogeneity within tumours. Lastly, even though these models lack the prediction of specific tumour-to-tissue infiltrative shape as expressed by the average surface distance, the MC–CC model can provide accurate short- and long-term predictions on a rat-specific basis. The MC–CC model provides a more complete description of in vivo C6 glioma growth in rats compared with the standard RD model.

**Ethics.** All experimental procedures were approved by Vanderbilt University’s Institutional Animal Care and Use Committee.

**Authors’ contributions.** D.A.H. performed the experimental work, data analysis, study design, and drafted the manuscript; J.A.W. and S.L.B. participated in data analysis, design of the study and drafted the manuscript; J.A.W. and S.L.B. participated in data analysis, design of the study and drafted the manuscript; J.A.W. and D.A.H. performed the experimental work, data analysis, study design, and drafted the manuscript; M.I.M., E.C.R., V.Q. and T.E.Y. participated in conceiving the study and drafted the manuscript.

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**References**


