

Supporting Material of the manuscript:

Mathematical Model of Oxygen Transport in Tuberculosis Granulomas

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Model Formulation

The steady-state one-dimensional oxygen mass balance within the avascular region of a spherical granuloma yields:

$$D_{O_2}^e \frac{1}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_{O_2}}{dr} \right) + Q_{O_2} = 0 \quad (1)$$

The volumetric net oxygen source term Q_{O_2} within the tissue is given by

$$Q_{O_2} = \psi_B C_{O_2,b} - \frac{kC_{O_2}}{1 + KC_{O_2}} \quad (2)$$

where the second term on the right hand side is the Michaelis-Menten (MM) form of cellular oxygen consumption, and the first term on the right represents the source of oxygen supply from the blood vessels, where ψ_B is the volumetric rate of oxygen delivery from the vasculature to the tissue².

Next, the following additional assumptions are made:

- 1) In the perfused region, i.e., in the sub-region between R_0 and R (Figure 2A), blood vessels exist so that the vascular source term, $\psi_B C_{O_2,b}$, in Eq. (2) is dominant in this sub-

region, i.e., $\psi_B C_{O_2,b} \gg \frac{kC_{O_2}}{1 + KC_{O_2}}$,

2) There are no blood vessels in the avascular region, i.e., for $r \leq R$, $\psi_B = 0$, so that

$$Q_{O_2} = -\frac{kC_{O_2}}{1 + KC_{O_2}}, \text{ i.e., } Q_{O_2} \text{ is simply a term for oxygen consumption.}$$

3) Any bacilli present in the granuloma are undergoing anaerobic respiration; therefore, MTB does not contribute to oxygen consumption in this model.

Thus the mass balance of oxygen takes the form:

$$\frac{D_{O_2}^e}{r^2} \frac{d}{dr} \left(r^2 \frac{dC_{O_2}}{dr} \right) = \frac{kC_{O_2}}{1 + KC_{O_2}} \quad (3)$$

subject to the boundary conditions:

$$\begin{aligned} \text{B.C. 1: at } r = R & \quad C_{O_2} = C_{O_2,b} \\ \text{B.C. 2: at } r = R_H & \quad C_{O_2} = C_{O_2,H} \\ \text{B.C. 3: at } r = R_H & \quad N_{O_2} = \text{continuous} \\ \text{B.C. 4: at } r = R_C & \quad C_{O_2} = C_{O_2,C} \\ \text{B.C. 5: at } r = R_C & \quad \frac{dC_{O_2}}{dr} = 0 \end{aligned} \quad (4)$$

which require further discussion. When the oxygen concentration drops due to cellular consumption to $C_{O_2} = C_{O_2,H}$ at some (as yet unknown) value of $r = R_H$ (B.C. 2), the cells become hypoxic. When the oxygen concentration drops further to $C_{O_2} = C_{O_2,C}$ at some (also unknown) value of $r = R_C$ (B.C. 4), the cells die and the oxygen consumption rate becomes zero throughout the necrotic core. Since the MM kinetics in its usual form is inconsistent with this last fact of zero cellular consumption at a finite oxygen concentration (Figure 3), it must be accounted for in the model by artificial means, i.e., B.C. 5, in which the concentration gradient at the boundary of the necrotic core, $r = R_C$, is forced to be zero.

The final boundary condition, B.C. 3, i.e., at $r = R_H$, the flux $N_{O_2} = \text{continuous}$, implies that the concentration gradient is continuous. This guarantees that even though $r = R_H$ marks the start of hypoxia, and, as assumed here, a change in cellular metabolism, there is no discontinuity in the concentration profile at this location, ascertained by matching the concentration gradient at this location between the different sub-regions (Figure 2B). This boundary condition also allows the estimation of the MM kinetic parameter K , as discussed below in the model parameters.

For ease of equation manipulation and subsequent numerical calculations, non-dimensionalization is applied based on the following dimensionless variables:

$$y = \frac{r}{R} ; f = \frac{C_{O_2}}{C_{O_2,b}} ; \phi = R \sqrt{\frac{k}{D_{O_2}^e}} ; \chi = KC_{O_2,b} \quad (5)$$

where ϕ is the so-called Thiele Modulus⁶, and is a measure of the ratio of the intrinsic rates of oxygen consumption and diffusion. Note that because constant physical properties are assumed in the idealized spherical granuloma, the only parameter in the definition of the Thiele Modulus that can vary is R , the radius of the avascular region. In other words, because k and $D_{O_2}^e$ are constant, the Thiele Modulus can be considered to be a dimensionless form of the avascular radius (and, by extension, a representation of unitless avascular granuloma size, where a large Thiele Modulus indicates a large granuloma).

In terms of the dimensionless parameters, thus, Eq. (3) may be written in the dimensionless form:

$$\frac{1}{y^2} \frac{d}{dy} \left(y^2 \frac{df}{dy} \right) = \phi^2 \frac{f}{1 + \chi f} \quad (6)$$

This is subject to the boundary conditions in Eq. (4), written as follows in dimensionless form

$$\begin{aligned}
\text{B.C. 1: at } y=1 & \quad f=1 \\
\text{B.C. 2: at } y=y_H & \quad f=f_H \\
\text{B.C. 3: at } y=y_H & \quad \left(\frac{df}{dy}\right)_{y_H} = \text{continuous} \\
\text{B.C. 4: at } y=y_C & \quad f=f_C \\
\text{B.C. 5: at } y=y_C & \quad \frac{df}{dy} = 0
\end{aligned} \tag{7}$$

Equation (6) represents the dimensionless second-order nonlinear ordinary differential equation for oxygen concentration in the avascular region within the granuloma with the full Michaelis-Menten (MM) form of oxygen consumption kinetics. It can be solved only numerically subject to two boundary conditions. The least restrictive boundary conditions are B.C. 1 and B.C. 5 (Eq. 7), i.e., the specification of bulk concentration of oxygen, and the concentration gradient being zero at the granuloma center ($y=y_C$). Using *Mathematica*[®] (Version 6.0, Wolfram Research, Inc., Champaign, IL) for the numerical solution of Eq. 6 subject to these two boundary conditions, the result is shown in Figure 3, with $\chi = KC_{O_2,b}$ and other parameters listed in Table 1 for various values of the Thiele Modulus, ϕ , a representation of granuloma size.

Since Eq. (6) can be solved only numerically, more insights can be gained by considering the zero- and first-order limiting cases of the MM kinetics that describe the approximate oxygen consumption in sub-regions I and II in a granuloma (Figure 2B), which allow for explicit solutions that can analytically describe the concentration profile.

Because predicting the emergence of hypoxia and necrosis in TB granulomas is of central importance in understanding the course of disease progression, it is desired to use this model to predict the corresponding radii, R_H and R_C , or, in dimensionless terms, $y_H = R_H / R$ and

$y_c = R_c / R$. The determination of these radii and the limiting solutions of the sub-regions I and II are discussed fully in the following sections.

Derivation of the Limiting Solutions

As shown in Figure 2B, the avascular region is further subdivided sub-regions I and II. It is assumed here that the change from zero- to first-order kinetics occurs at $r = R_H$, where $C_{O_2} = C_{O_2,H}$, rationalized on the basis of altered cell metabolism in response to local oxygen concentration. This concentration, $C_{O_2,H}$, is taken as that indicated by a positive pimonidazole stain (Figure 1).

Avascular Sub-Region II with First-Order Kinetics, $R_c \leq r \leq R_H$

For the region where first-order kinetics is presumed to exist, $\chi f \ll 1$, i.e., $Q_{O_2} = -kC_{O_2}$, Eq. (6) becomes

$$\frac{1}{y^2} \frac{d}{dy} \left(y^2 \frac{df}{dy} \right) = \phi^2 f \quad (8)$$

This linear second-order ordinary differential equation is subject to the boundary conditions

$$\begin{aligned} \text{B.C. 2: at } y = y_H & \quad f = f_H \\ \text{B.C. 4: at } y = y_c & \quad f = f_c \\ \text{B.C. 5: at } y = y_c & \quad \frac{df}{dy} = 0 \end{aligned} \quad (9)$$

The resulting solution for the dimensionless concentration profile subject to B.C. 4 and B.C. 5, after considerable manipulation and use of identities of hyperbolic functions, is

$$f = \frac{f_c}{\phi y} \left[\phi y_c \cosh \{ \phi (y - y_c) \} + \sinh \{ \phi (y - y_c) \} \right] \quad (10)$$

This solution is valid in the region $y_c \leq y \leq y_H$. However, in Eq. (10), y_c is still unknown and is, in fact, a key goal.

Further, using B.C. 2 in Eq. (10)

$$f_H = \frac{f_C}{\phi y_H} \left[\phi y_C \cosh\{\phi(y_H - y_C)\} + \sinh\{\phi(y_H - y_C)\} \right] \quad (11)$$

which interrelates y_C and y_H for known $f_C (= C_{O_2,C} / C_{O_2,b})$ and $f_H (= C_{O_2,H} / C_{O_2,b})$, and for a given value of the Thiele Modulus, ϕ , which is essentially the dimensionless particle radius.

If y_H could be determined, i.e., via zero-order analysis of sub-region I, as discussed in the following section, then the dimensionless necrotic core radius, y_C , can be determined.

Avascular Sub-Region I with Zero-Order Kinetics, $R_H \leq r \leq R$

For sub-region I, where zero-order kinetics is presumed to exist, $\chi f \gg 1$, so that 1 in the denominator of Eq. (6) may be neglected, hence reducing it to

$$\frac{1}{y^2} \frac{d}{dy} \left(y^2 \frac{df}{dy} \right) = \frac{\phi^2}{\chi} \quad (12)$$

Integrating twice and using B.C. 1 to determine one of the two constants of integration

$$f = 1 - \frac{\phi^2}{6\chi} (1 - y^2) + C_1 \left(1 - \frac{1}{y} \right) \quad (13)$$

However, the constant C_1 is yet to be determined, for which B.C. 3 may be used, i.e., at $y = y_H$, $df/dy = \text{continuous}$, or that the concentration gradient is continuous for the first-order and zero-order solutions. Thus, we need to equate at $y = y_H$ the dimensionless concentration gradient of the zero-order solution for sub-region I

$$\left(\frac{df}{dy} \right)_{y_H} = \frac{\phi^2}{3\chi} y_H + \frac{C_1}{y_H^2} \quad (14)$$

with the corresponding concentration gradient for first-order solution for sub-region II obtained by differentiating Eq. (10) and using at $y = y_H$

$$\left(\frac{df}{dy}\right)_{y_H} = \frac{f_C}{y_H^2} \left[(y_H - y_C) \cosh\{\phi(y_H - y_C)\} + (y_H \phi y_C - 1/\phi) \sinh\{\phi(y_H - y_C)\} \right] \quad (15)$$

Equating the above two expressions to find C_1 and the using it in Eq. (13) provides the concentration profile for sub-region II

$$f = 1 - \frac{\phi^2}{6\chi} (1 - y^2) + \left\{ f_C [(y_H - y_C) \cosh\{\phi(y_H - y_C)\} + (y_H \phi y_C - 1/\phi) \sinh\{\phi(y_H - y_C)\}] - \frac{\phi^2}{3\chi} y_H^3 \right\} \left(1 - \frac{1}{y} \right) \quad (16)$$

However, we are primarily interested in determining y_C and y_H , for which one relation (Eq. 11) is already available. To obtain a second relation between these unknown quantities, we evaluate Eq. (16) at $y = y_H$ and rearrange

$$1 - f_H = \frac{\phi^2}{6\chi} (1 - y_H^2) + \left\{ f_C [(y_H - y_C) \cosh\{\phi(y_H - y_C)\} + (y_H \phi y_C - 1/\phi) \sinh\{\phi(y_H - y_C)\}] - \frac{\phi^2}{3\chi} y_H^3 \right\} \left(\frac{1}{y_H} - 1 \right) \quad (17)$$

In summary, Eqs. (11) and (17) provide the relations between y_C and y_H , and these two equations can be analytically solved simultaneously to find the roots y_C and y_H for known f_C and f_H , and for a given Thiele Modulus, ϕ (using *Mathematica*[®]), as shown in Figure 4. With the value of ϕ_m (i.e., minimum granuloma size with necrosis), determined as described below in kinetic parameters, y_C and y_H are plotted as a ratio of ϕ/ϕ_m (i.e., dimensionless normalized granuloma size) in Figure 4. Figure 4 also includes the theoretical values for $y_H - y_C$, or the dimensionless thickness of the hypoxic region, versus ϕ/ϕ_m .

The theoretical dimensionless values of y_C and y_H from Figure 4 can be converted to dimensional values from the avascular granuloma radius, R , using the definition of the Thiele

Modulus ($\phi = R\sqrt{k/D_{O_2}^e}$), and the parameter values of k and $D_{O_2}^e$, given in Table 1. These results are compared to experimentally measured values of R_C and R_H in Figure 5, quantified via image analysis methods discussed below. Furthermore, oxygen concentration profiles can be plotted for various values of ϕ , as shown in Figure 6, by solving Eqs. (10) and (16).

Model Parameters

Physical Parameters

The two main relevant physical parameters (which are assumed to be constant) are the effective diffusivity of oxygen in the interstitial fluid of the granuloma tissue, $D_{O_2}^e$, and the Henry's Law constant for the solubility of oxygen in the interstitial fluid (i.e., the medium in which cells and extracellular matrix materials exist in the interstitial space, which is often estimated to be roughly identical to plasma), H_{O_2} (where $C_{O_2} = H_{O_2}p_{O_2}$). Here, we adopt $H_{O_2} = 1.34 \times 10^{-9}$ mol O_2/cm^3 -mmHg as an appropriate value for the Henry's Law constant for oxygen⁹.

In biological tissues, further, the value of D_{O_2} does not appear to vary widely; literature values of oxygen diffusivity in various tissues ranges from $1.9 - 2.8 \times 10^{-5}$ cm^2/s ^{3, 7, 9}. Consequently, a middle-range value of 2.5×10^{-5} cm^2/s has simply been adopted here; based on the range, the chosen value is within a $\sim 20\%$ error margin.

Relevant Oxygen Concentrations in Granulomas

We have published oxygen tension values measured with an oxygen-sensitive electrode in the normal (uninfected) lung tissue and granuloma necrotic cores of TB-infected rabbit lungs¹⁰, and have assumed here that the hypoxic regions of correspond to the literature value of known $pO_{2,H}$; these values are employed here as: 1) $pO_{2,b} = 60$ mmHg, 2) $pO_{2,H} = 10$ mmHg, and 3) $pO_{2,C} = 2$

mmHg. As discussed below in the experimental procedures, the hypoxic partial pressure $pO_{2,H} = 10\text{mmHg}$ is well documented in the literature as the oxygen tension at which positive pimonidazole stain occurs, which is used here as the indicator of tissue hypoxia.

Adopting the interstitial fluid Henry's law constant $H_{O_2} = 1.34 \times 10^{-9} \text{ mol O}_2/\text{cm}^3\text{-mmHg}$ ⁹, these correspond to the following concentrations: 1) $C_{O_2,b} = 8.04 \times 10^{-8} \text{ mol O}_2/\text{cm}^3$, 2) $C_{O_2,H} = 1.34 \times 10^{-8} \text{ mol O}_2/\text{cm}^3$, and 3) $C_{O_2,c} = 2.68 \times 10^{-9} \text{ mol O}_2/\text{cm}^3$. Consequently, the corresponding dimensionless concentrations $f_H = C_{O_2,H} / C_{O_2,b} = 0.167$, and $f_C = C_{O_2,c} / C_{O_2,b} = 0.033$.

Kinetic Parameters

The remaining MM kinetic parameters, k and K , are determined as follows. The model permits prediction of the minimum size of the avascular granuloma particle, R_{\min} , which would have the beginnings of a necrotic core within it. Clearly, when the granuloma is small enough, the oxygen concentration throughout remains above $C_{O_2,c}$. At a particular granuloma size, R_{\min} , the oxygen concentration at $y=0$ ($r = 0$) reaches $C_{O_2,c}$. Experimental evidence from an independent cohort of tissues from untreated TB-infected rabbits (unpublished) provides an $R_{\min} = 0.308 \text{ mm}$.

As further mentioned above, since k and $D_{O_2}^e$ are fixed based on the assumption of constant physical properties, the Thiele Modulus ϕ represents dimensionless granuloma size; therefore, a dimensionless minimum granuloma size with an emerging necrotic core can be defined as ϕ_m . When the modulus is small ($\phi < \phi_m$), there is no necrosis, as is known

experimentally. At a particular granuloma size, ϕ_m , necrosis first appears; for such a granuloma, $y_C = 0$. With this, Eq. (11) reduces to

$$f_H = f_C \frac{\sinh(\phi_m y_H)}{(\phi_m y_H)} \quad (18)$$

which has two unknowns, ϕ_m , corresponding to R_{\min} , and y_H . Thus, a second relation is needed between ϕ_m and y_H , which may be obtained from Eq. (17) with $y_C = 0$, which reduces to

$$1 - f_H = \frac{\phi_m^2}{6\chi} (1 - y_H^2) + \left\{ f_C [y_H \cosh(\phi_m y_H) - 1 / \phi_m \sinh(\phi_m y_H)] - \frac{\phi_m^2}{3\chi} y_H^3 \right\} \left(\frac{1}{y_H} - 1 \right) \quad (19)$$

Thus ϕ_m and y_H may be obtained from these two relations for given f_H and f_C . This value was determined to be $\phi_m = 6.9$. Using the corresponding experimental $R_{\min} = 0.308$ mm, along with the assumed $D_{O_2}^e = 2.5 \times 10^{-5}$ cm²/s, the value of $k = 1.09$ s⁻¹ may be calculated from $\phi_m = R_{\min} \sqrt{k / D_{O_2}^e}$. The accuracy in this value is also thus the same as that in value of D_{O_2} , i.e., $\pm 20\%$.

Finally, the MM kinetic parameter K is obtained as follows. It is hypothesized here that the change from zero- to first-order oxygen consumption kinetics occurs when the cells become hypoxic, i.e., at $C_{O_2,H}$. From the physiological (and mathematical) boundary condition at this oxygen concentration (B.C. 3), and the limiting forms of the MM kinetics, the rate of oxygen consumption is continuous at this point, i.e.,

$$-Q_{O_2} = \underbrace{\frac{k}{K}}_{\text{zero-order}} = \underbrace{k C_{O_2,H}}_{\text{first-order}} \quad (20)$$

Therefore, $K = 1/C_{O_2,H} = 1/K_M$, the MM constant. This is a physiologically relevant way to estimate K , and the value calculated is $7.46 \times 10^7 \text{ cm}^3/\text{mol O}_2$, which is similar to a range of values observed for various tissues^{3,7}. The model parameters thus estimated are summarized in Table 1.

Experimental Procedures

Animal Experiments

The experimental results reported in this paper were gathered from historical control tissues from female New Zealand White rabbits from our previously performed animal studies⁴; as described above, the kinetic parameter k was calculated from an independent cohort of tissues (unpublished). Briefly, female rabbits were infected with *Mycobacterium tuberculosis* HN878 through an aerosol inoculation system that delivered approximately 200 colony-forming units (CFU)/liter PBS (phosphate buffered saline) diluting solution to each rabbit. The rabbits were allowed to undergo natural disease progression for about 10 weeks until a chronic infection state was established. During early infection, it is common for many small granulomas to emerge and then resorb quickly due to the robustness of the rabbit immune system; by the late stage of infection, most of the initial granulomas have disappeared, while those that remain during chronic infection are relatively static in size and activity. Thus, the granulomas used for comparison to the theoretical model are assumed to be at steady-state, i.e., there is no change in granuloma size at this time point in the infection. Post-treatment, animals were sedated, euthanized and necropsied, and full lung sections and/or individual granulomas were resected and stored in either paraffin wax or frozen blocks⁴.

Pimonidazole Stain

Rabbit lung sections were then sectioned and stained with pimonidazole. Pimonidazole hydrochloride (HypoxyprobeTM-1; Hypoxyprobe, Inc., Burlington, MA) is an imaging agent that is bioreductively activated under hypoxic conditions in mammalian tissues; a positive stain for hypoxia stains dark brown in immunohistochemical (IHC) staining, i.e., brightfield, images. Pimonidazole is able to diffuse across cell membranes and, depending on the amount of local oxygen, will undergo conjugation, oxidation, or reduction. In mammalian tissues with partial pressures of $pO_2 \leq 10$ mmHg, pimonidazole is bioreductively activated by nitroreductases, after which it binds to and forms stable covalent adducts with thiol (sulfhydryl) groups on proteins^{1,5,8}. Rat experiments by Arteel et al. (1998) demonstrate that the rate of reductive metabolism of pimonidazole is regulated by cellular oxygen tension.

Pimonidazole residing in tissues at the time of necropsy remains bound thiol-containing molecules when the dissected tissues become anoxic, and are then detected by antibodies that bind to the pimonidazole protein adduct. In the animal experiments, the rabbits were given 30 mg/kg i.v. pimonidazole for 16 to 20 hours prior to necropsy. For detection of pimonidazole adducts in the tissues, paraffin sections were deparaffinized, incubated with the HypoxyprobeTM-1 antibody, followed by a biotin-conjugated goat antimouse immunoglobulin G antibody, and the DAB (3,3-diaminobenzidine) labeling system, as described previously⁴.

Image Analysis

Image analysis was performed in ImageJ (open-source software, National Institutes of Health), from IHC images were scanned using a Nanozoomer 2.0 HT C9600 Series at a resolution matching a micron to pixel ratio of 0.23 (40x magnification equivalent). Once the granuloma

images were obtained, the next issue was to find an appropriate descriptor of the equivalent radius R for transport modeling in a spherical equivalent of a non-spherical granuloma particle. From among the various descriptors considered for this, it was determined here that the radius R of the largest circle that can be completely enclosed within a granuloma is the most relevant descriptor of the equivalent sphere model for diffusion and reaction, as it represents the maximum depth of penetration of a diffusing solute. Therefore, largest enclosed circles were drawn to determine four relevant radii: 1) outer granuloma radius, R_0 , 2) avascular radius, R , 3) hypoxic radius, R_H , and 4) necrotic radius, R_C . From the control (untreated) animals of our previous experiment⁴, there were 11 granulomas that were well-defined and necrotic, and thus, applicable to this type of analysis. The measured radii are summarized in Supplementary Table 1.

Supplementary Table

Table S1. Radial measurements of necrotic (R_C), hypoxic (R_H), avascular (R), and total (R_0) granuloma radii, as well as the hypoxic region thickness ($R_H - R_C$), from the experimental rabbit model (mm).

R_0	R	R_H	R_C	$R_H - R_C$
0.393	0.296	0.206	0.162	0.045
0.466	0.299	0.213	0.163	0.050
0.482	0.322	0.223	0.165	0.058
0.498	0.434	0.375	0.339	0.036
0.543	0.404	0.258	0.146	0.112
0.579	0.477	0.276	0.187	0.090
0.615	0.428	0.219	0.119	0.100
0.629	0.542	0.312	0.220	0.092
0.666	0.570	0.419	0.364	0.055
0.695	0.613	0.501	0.420	0.080
0.742	0.543	0.390	0.332	0.058

Glossary of Terms

Nomenclature

C_{O_2}	concentration of oxygen (mol/cm ³)
$C_{O_2,b}$	constant bulk concentration of oxygen in the perfused region (mol/L)
$C_{O_2,c}$	constant critical concentration of oxygen at the boundary of and throughout the necrotic core (mol/L)
$C_{O_2,H}$	concentration of oxygen at which the pimonidazole stain is positive, indicating hypoxia (mol/L)
$D_{O_2}^e$	effective diffusion coefficient of oxygen in the interstitial fluid (mm ² /s)
f	dimensionless concentration of oxygen
f_c	dimensionless critical concentration of oxygen
f_H	dimensionless hypoxic concentration of oxygen
H_{O_2}	Henry's law constant for oxygen solubility (mol/cm ³ -mmHg)
k	first-order rate constant for oxygen consumption (s ⁻¹)
K	inverse of the half-saturation Michaelis-Menten constant (L/mol)
p_{O_2}	partial pressure of oxygen (mmHg)
$p_{O_2,b}$	bulk partial pressure of oxygen (mmHg)
$p_{O_2,c}$	critical partial pressure of oxygen (mmHg)
$p_{O_2,H}$	hypoxic partial pressure of oxygen (mmHg)
Q_{O_2}	oxygen generation rate (mol/mm ³ s)

r	radial position in the granuloma
R	radius of avascular region (mm)
R_0	radius of the granuloma (mm)
R_{\min}	minimum granuloma particle size at which necrosis emerges (mm)
R_C	radius of the necrotic core (mm)
R_H	radius of the hypoxic region (mm)
y	dimensionless radial position in the avascular region
y_C	dimensionless radius of the necrotic core
y_H	dimensionless radius of the hypoxic region

Greek Symbols

χ	dimensionless Michaelis-Menten kinetic factor
ϕ	Thiele modulus
ψ_B	volumetric rate of oxygen delivery from the vasculature into the tissue (mm ³ /s)
ϕ_m	dimensionless minimum granuloma particle size at which necrosis emerges

Supplementary References

1. Arteel G. E., R. G. Thurman and J. A. Raleigh. Reductive metabolism of the hypoxia marker pimonidazole is regulated by oxygen tension independent of the pyridine nucleotide redox state. *Eur J Biochem* 253: 743-750, 1998.
2. Baxter L. T. and R. K. Jain. Transport of fluid and macromolecules in tumors. I. Role of interstitial pressure and convection. *Microvasc Res* 37: 77-104, 1989.
3. Chen J., A. T. Layton and A. Edwards. A mathematical model of O₂ transport in the rat outer medulla. I. Model formulation and baseline results. *Am J Physiol Renal Physiol* 297: F517-536, 2009.
4. Datta M., L. E. Via, W. S. Kamoun, C. Liu, W. Chen, G. Seano, D. M. Weiner, D. Schimel, K. England, J. D. Martin, X. Gao, L. Xu, C. E. Barry, 3rd and R. K. Jain. Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. *Proc Natl Acad Sci U S A* 112: 1827-1832, 2015.
5. Durand R. E. and J. A. Raleigh. Identification of nonproliferating but viable hypoxic tumor cells in vivo. *Cancer Res* 58: 3547-3550, 1998.
6. Fogler H. S. *Elements of Chemical Reaction Engineering*. Upper Saddle River, NJ: Prentice Hall, 2006.
7. Golub A. S. and R. N. Pittman. Oxygen dependence of respiration in rat spinotrapezius muscle in situ. *Am J Physiol Heart Circ Physiol* 303: H47-56, 2012.
8. Raleigh J. A., D. P. Calkins-Adams, L. H. Rinker, C. A. Ballenger, M. C. Weissler, W. C. Fowler, Jr., D. B. Novotny and M. A. Varia. Hypoxia and vascular endothelial growth factor expression in human squamous cell carcinomas using pimonidazole as a hypoxia marker. *Cancer Res* 58: 3765-3768, 1998.
9. Truskey G. A., F. Yuan and D. F. Katz. *Transport phenomena in biological systems*. Upper Saddle River, NJ: Pearson/Prentice Hall, 2004.
10. Via L. E., P. L. Lin, S. M. Ray, J. Carrillo, S. S. Allen, S. Y. Eum, K. Taylor, E. Klein, U. Manjunatha, J. Gonzales, E. G. Lee, S. K. Park, J. A. Raleigh, S. N. Cho, D. N. McMurray, J. L. Flynn and C. E. Barry, 3rd. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. *Infect Immun* 76: 2333-2340, 2008.