Microvascular transport model predicts oxygenation changes in the infarcted heart after treatment

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Wang B, Scott RC, Pattillo CB, Prabhakarpandian B, Sundaram S, Kiani MF. Microvascular transport model predicts oxygenation changes in the infarcted heart after treatment. Am J Physiol Heart Circ Physiol 293: H3732–H3739, 2007. First published October 19, 2007; doi:10.1152/ajpheart.00735.2007.—Chronic heart failure is most commonly due to ischemic cardiomyopathy after a previous myocardial infarction (MI). Rebuilding lost myocardium to prevent heart failure mandates a neovascular network able to nourish new cardiomyocytes. Previously we have used a series of novel techniques to directly measure the ability of the scar neovasculature to deliver and exchange oxygen at 1–4 wk after MI in rats following left coronary artery ligation. In this study, we have developed a morphologically realistic mathematical model of oxygen transport in cardiac tissue to help in deciding what angiogenic strategies should be used to rebuild the vasculature. The model utilizes microvascular morphology of cardiac tissue based on available morphometric images and is used to simulate experimentally measured oxygen levels after MI. Model simulations of relative oxygenation match experimental measurements closely and can be used to simulate distributions of oxygen concentration in normal and infarcted rat hearts. Our findings indicate that both vascular density and vascular spatial distribution play important roles in cardiac tissue oxygenation after MI. Furthermore, the model can simulate relative changes in tissue oxygen levels in infarcted tissue treated with proangiogenic compounds such as losartan. From the minimum oxygen concentration myocytes need to maintain their normal function, we estimate that 2 wk after MI 29% of the myocardium is severely hypoxic and that the vascular density of the infarcted tissue should reach 75% of normal tissue to ensure that no areas of the myocardium are critically hypoxic. 

HEART FAILURE is a major health problem of worldwide proportions. A transmural myocardial infarction (MI) involves a loss of necrotic cardiomyocytes and a proteolysis of extracellular matrix, coronary vasculature, and adrenergic nerves (49). Subsequent tissue repair includes the formation of an infarct scar, which restores structural integrity to the infarct site; it does not however, involve a significant regeneration of cardiomyocytes. The infarct scar is composed of a fibrillar collagen meshwork with intertwining postganglionic sympathetic neurons and a population of myofibroblasts nourished by a neovasculature (50). This newly formed infarct scar is not equipped to return the heart to normal myocardial function. Approaches such as stem cell therapy and/or targeted delivery of proangiogenic compounds to rebuild lost myocardial tissue, including its vasculature, represent innovative strategies that may prevent the appearance of chronic cardiac failure after MI (11, 33, 34, 38, 57). However, cardiomyocytes are highly dependent on oxygen for their survival (51), and a rebuilt myocardium must include a vascular network able to nourish new cardiomyocytes under diverse physiological conditions. Currently, it is not clear whether treatments such as stem cell therapy can produce the appropriate neovasculature to be able to support these obligate aerobic cells. Proangiogenic strategies, such as a novel method recently developed in our laboratory for targeting drug carriers to infarct tissue (43), may be required to rebuild the vasculature in conjunction with treatments such as stem cell therapy.

A two-pronged approach is required to develop a rational method for regenerating the microvasculature after MI. First, perfusion and tissue hypoxia should be simultaneously and directly measured. Second, it should be determined how many vessels myocardial fibers need and how these vessels should be distributed to deliver enough oxygen to maintain their normal function. To achieve the first goal, we have adapted a series of methods from the field of tumor biology to directly measure the number of anatomic and perfused vessels and the level of hypoxia after MI (56). In this approach, 3,3′-dihexyloxyacarbocyanine iodide (DiOC6) and CD31 staining are used to measure the number of anatomic and perfused vessels, respectively. Tissue hypoxia is directly measured from immunohistochemical staining for an antibody to the adduct of EFS; EFS is a pentfluorinated derivative of etanidazole that is preferentially metabolized in hypoxia cells (27). These findings are then used to study myocardial oxygenation and its relationship with the number of anatomic and perfused vessels (56).

As for the second goal, mathematical modeling offers the only realistic hope for systematically determining the number and distributions of vessels that are needed for proper oxygenation of infarcted tissue. Traditionally Krogh cylinder-based geometric models (7, 26) have been used to study oxygen transport from vessels to muscular tissue. These simplified models are easy to understand and beneficial for studying the overall oxygen distribution in the tissue, but they are unable to give detailed and accurate information on oxygen distribution patterns. In recent years more sophisticated mathematical models of oxygen delivery to tissue have been developed (16, 17, 19, 20, 37, 44). However, in part because of the lack of detailed experimental findings in an infarcted tissue, many of these models cannot be validated experimentally in an infarct tissue model and as such may have limited application in guiding...
experimental design. An alternative approach may be needed to determine the number and distribution of vessels required for proper functioning of postinfarct tissue with a mathematical model that can be validated against currently available experimental data.

We have developed and validated a mathematical model to quantitatively analyze the experimentally obtained oxygenation levels in tissue sections and elucidate their relationship with number and distribution of perfused vessels. This model is able to accurately predict tissue oxygenation in post-MI tissue after various experimental and simulated interventions, and can then be used to answer the question of how many vessels the myocardial fibers in the MI region need and how these vessels should be distributed to prevent tissue hypoxia.

**METHODS**

**Animal model of myocardial infarction.** As described previously (54), a series of novel techniques were used to characterize tumor vascular larity, perfusion, and levels of hypoxia (13, 27) were adapted to quantify the components and functionality (i.e., ability to deliver oxygen) of the scar vascular network at 1–4 wk after MI. In brief, an anterior transmural MI was induced in Sprague-Dawley rats by ligation of the left coronary descending artery. At 1–4 wk after MI, rats were sedated and EFS was injected. Six hours later, DiOC₃ was injected. One minute later, hearts were removed, frozen, and sectioned (10-μm-thick sections). CD31 staining was used to visualize anatomic vessels and to calculate the diameters of vessels. Fluorescent staining with DiOC₃ was used to visualize patent vessels with blood flow in infarcted tissue, which was defined as the border zone of the necrotic area. Tissue hypoxia was quantified with EF5, a nitroheterocyclic compound that has been shown to form adducts at a much higher rate in hypoxic tissue (27). EF5 has been shown to reach well-perfused as well as poorly perfused areas of the tissue by diffusion from the microcirculation, and the intensity of EF5/Cy3 has been shown to be inversely proportional to the degree of tissue oxygenation (12). This combination of fluorescent and immunohistological stains was then used to define the distribution of distances from cells to the nearest anatomic or perfused vessel. The protocol was approved by the Animal Care and Use Committee of Temple University.

**Mathematical model of myocardial oxygenation in MI rat heart.** A two-dimensional (2D) model of physiological oxygen transport in tissue was developed with the finite-volume Computational Fluid Dynamics (CFD) code CFD-ACE+ (ESI-CFD, Huntsville, AL), on a standard desktop personal computer. The CFD code uses fast and efficient numerical methods tailored to solve physiological transport problems in realistic geometries. The solver is based on a finite-volume, pressure-based, strongly conservative formulation. An iterative solution procedure based on the SIMPLEC algorithm (35) is used to obtain a converged solution for simulated parameters. The method is based on a continuous representation of the tissue around the vessels in which all tissue components (myocyte, vessel wall, and interstitial space) are modeled as a homogeneous and continuous material. Furthermore, the diffusion of oxygen in the blood is not considered in this model, and the model calculates the oxygen diffusion from the vessel wall to the tissue. We assume that oxygen binding and unbinding with myoglobin occur so rapidly that the myoglobin binding term can be ignored (5). This provides a fast and efficient way of determining the effect of changes in oxygen concentration and vessel density on a macroscale in both normal and diseased myocardium. In addition, this approach allows us to quantitatively analyze our experimental data. The CFD model provides a convenient methodology for characterizing and studying transport and biochemical/metabolic reactions simultaneously.

**Transport model.** Assuming no perfusion and a steady-state calculation, the generalized mass conservation equation for oxygen transport in the tissue reduces to \( \text{DO}_2 \text{CO}_2 = \text{VO}_2 \), where \( \text{DO}_2 \) is oxygen diffusivity in tissue, \( \text{CO}_2 \) is molar concentration of oxygen, and \( \text{VO}_2 \) is the consumption rate.

**Oxygen consumption model.** Oxygen consumption was assumed to be homogeneously distributed within the homogeneous tissue according to Michaelis-Menten kinetics (5): \( \text{VO}_2 = \text{VO}_{2\text{SM}} \text{CO}_2/(\text{CO}_2 + K_m) \), where \( \text{VO}_{2\text{SM}} \) is defined as the maximal rate of oxygen consumption, \( \text{CO}_2 \) is oxygen concentration in the tissue, and \( K_m \) is the effective Michaelis-Menten constant (6). Matrix-based, stiff kinetics solvers were employed for the reaction kinetics. A second-order central differencing method was used for spatial interpolation of concentration variables, and Euler time integration was employed. All data were postprocessed in CFD-VIEW visualization software.

**Boundary conditions.** The vessels served as inlets with the condition of fixed initial oxygen concentration (\( \text{CO}_{2\text{in}} \)), and a zero flux at the external tissue boundary was imposed based on consideration of symmetry. Therefore, the only flow of oxygen into the tissue components was by diffusion due to the gradient of the oxygen concentration.

The delivery of oxygen to the tissue components from blood as initial oxygen concentration (\( \text{CO}_{2\text{in}} \)) is determined by two factors: coronary blood flow and oxygen content in the blood. Oxygen concentrations (\( \text{CO}_{2\text{in}} \)) in the vessels at each time after MI, as listed in Table 1, are calculated, using published methods (22, 28) to account for changes in coronary blood flow with time after MI (1, 10, 15, 21, 40).

**Initial conditions.** In the present model, blood flow in the vessels at a given time point after MI is assumed to be steady and the oxygen is driven into the tissue components with a constant boundary condition (\( \text{CO}_{2\text{in}} \)). The model is then used to simulate the long-term, steady-state distribution of oxygen in the tissue. As an initial condition, oxygen concentration everywhere in the tissue is set at zero; steady-state tissue oxygen concentration is then calculated based on the balance of the vascular source and tissue metabolic sinks.

**Grid/parament values.** For all the simulations, a 2D geometry was used. Unstructured numerical grids were created with the geometry/CAD software CFD-GEOM with the DXF file created from the images. The computational domain of the created geometry (Fig. 1, middle) comprised grid cells (Fig. 1, right) ranging from 500,000 to 2,000,000 depending on the size of the network. The diameter of myocytes ranged from 15 to 25 μm (4), and the average grid size used for the computational studies was 2 μm.

In the tissue compartment, which is assumed to be a homogeneous combination of all the tissue components (myocyte, interstitial space, and endothelial cells), oxygen diffusivity (39, 52) and maximal \( \text{O}_2 \) consumption rate (5) values from rat cardiac muscle were used. In the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Numerical Value</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>( \text{O}<em>2 ) maximal consumption rate ( \text{VO}</em>{2\text{SM}} )</td>
<td>200 μL-min⁻¹.g⁻¹</td>
<td>5</td>
</tr>
<tr>
<td>Hemoglobin concentration in blood (Hb)</td>
<td>15 g/dl</td>
<td>18,31</td>
</tr>
<tr>
<td>( \text{CO}<em>2 ) saturation (( \text{S}</em>{\text{O}_2} ))</td>
<td>45.6%</td>
<td>5,30</td>
</tr>
<tr>
<td>( \text{O}_2 ) diffusivity in tissue (( \text{DO}_2 ))</td>
<td>2.41 ( \times 10^{-8} ) cm²/s</td>
<td>39,52</td>
</tr>
<tr>
<td>( \text{O}<em>2 ) partial pressure (( \text{P}</em>{\text{O}_2} ))</td>
<td>28.6 mmHg</td>
<td>9</td>
</tr>
<tr>
<td>( \text{O}_2 ) solubility</td>
<td>1.5 ( \times 10^{-8} ) M/mmHg</td>
<td>5</td>
</tr>
<tr>
<td>Myocyte ( \text{O}<em>2 ) solubility (( \text{a}</em>{\text{O}} ))</td>
<td>1.74 ( \times 10^{-6} ) M/mmHg</td>
<td>5</td>
</tr>
<tr>
<td>( \text{O}_2 ) consumption constant (( K_m ))</td>
<td>( \text{a}_{\text{O}} \times (0.5 \text{mmHg}) )</td>
<td>5,30</td>
</tr>
<tr>
<td>( \text{O}<em>2 ) initial concentration (( \text{CO}</em>{2\text{in}} )) Normal</td>
<td>66.56 μM</td>
<td>40</td>
</tr>
<tr>
<td>1 wk after MI</td>
<td>57.24 μM</td>
<td>15</td>
</tr>
<tr>
<td>2 wk after MI</td>
<td>51.92 μM</td>
<td>1</td>
</tr>
<tr>
<td>3 wk after MI</td>
<td>45.26 μM</td>
<td>21</td>
</tr>
<tr>
<td>4 wk after MI</td>
<td>26.62 μM</td>
<td>10,40</td>
</tr>
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**MI, myocardial infarction.**
In the experimental studies, the EF5 intensity values in the normal as well as the infarcted tissue vary slightly in each batch of slides that are stained. Therefore, EF5 intensity values were normalized by dividing each pixel intensity in the infarct region by the average intensity in the normal tissue. In the modeling studies, intensity values in the normal tissue did not vary significantly, but to be able to compare model results with experimental data, we also used the intensity ratio, obtained by the same normalization. With this information the model results were compared with the experimental data previously derived from the EF5/Cy3 image (which shows the hypoxia levels) intensities of the infarcted rat heart.

The critical Po2 value below which the cardiac tissue is severely hypoxic may be a matter of debate given the fact that normal Po2 values, above which the myocyte is sufficiently oxygenated and shows neither physiological nor biochemical signs of hypoxia, are reported to range from 4 to 14 mmHg (2, 24, 48). In the present study we define severe hypoxia as Po2 ≤ 2 mmHg (58).

The mathematical model developed in this study was used to simulate oxygen concentrations in control and 1-, 2-, 3-, and 4-wk post-MI tissue in both morphological (3 networks per time point) and simulated (3 networks per time point) 2D geometries. For each time point we obtained three different networks from three different rats. Differences between experimental data and modeling data were tested by a multifactor analysis of variance (ANOVA) using Statgraphics Plus (Manugistics, Rockville, MD). Data are presented as means ± SE, and differences between the means are considered statistically significant if P < 0.05.

RESULTS

Previously we reported (56) that in postinfarct myocardium an increase in hypoxia levels is accompanied by a decrease in the number of perfused vessels. At the infarct site there was not only a 42% (1 wk after MI) to 75% (4 wk after MI) decrease in the number of anatomic vessels compared with controls but also a 53% (1 wk after MI) to 82% (4 wk after MI) decrease in the number of perfused vessels compared with controls. The decrease in the number of anatomic and perfused vessels after MI was accompanied by a significant and progressive increase in the distance to the nearest perfused vessel and the level of hypoxia (>100% increase in EF5/Cy3 staining). In normal myocardium, nearly 100% of tissue was within 20 μm of the nearest perfused vessel, while at 4 wk after MI only 40% of the tissue was within this distance. The scar neovascularure that normally appears after MI is not adequate to accommodate the metabolic demands of the existing tissue and must be augmented to support the rebuilt myocardium (49).
The predicted data from our mathematical model were compared with the experimental data from our previous study. As an example, Fig. 2 shows modeling results and experimental results from experimentally obtained morphological 2D vascular geometries from the MI area and normal area of an infarcted rat heart. Figure 2, A–C, are from the MI area of the infarcted rat heart, while Fig. 2, D–F, are from the normal area of infarcted rat hearts. Figure 2, A and D, are experimentally obtained morphological 2D vascular geometries (Fig. 2A shows the MI region, which is not highly vascularized, while Fig. 2D shows a highly vascularized normal region). Figure 2, B and E, are the oxygen levels as predicted by our mathematical model. Figure 2, C and F, are experimentally obtained results of hypoxia levels. In the modeling data (Fig. 2, B and E), red colors represent higher levels of hypoxia (lower oxygen concentrations), while blue colors represent lower levels of hypoxia (higher oxygen concentrations). In the experimental data (Fig. 2, C and F) the intensity of the red color represents higher levels of hypoxia (low oxygen concentrations). As shown in Fig. 2, for both modeling and experimental data the overall intensity of the MI region (Fig. 2, B and C) is much higher than that of the normal region (Fig. 2, E and F). There is good qualitative agreement between experimentally measured levels of hypoxia and those predicted by our mathematical model.

Experimentally we observed an inverse relationship between levels of hypoxia and the number of perfused vessels after MI such that as the number of perfused vessels decreases, the level of tissue hypoxia increases (56). The level of tissue hypoxia was normalized and recorded as the ratio of intensity in the infarcted region to the intensity in the normal region. We also calculated the levels of tissue hypoxia with our mathematical model and compared the results with the experimental data. As shown in Fig. 3, throughout the 1–4 wk after MI, the progressive and significant increase in experimentally measured tissue hypoxia (expressed as the ratio of hypoxia levels in infarcted over normal tissues) was successfully predicted by our mathematical model, i.e., there was no significant difference \(P > 0.05\) between experimentally observed and mathematically predicted levels of hypoxia in post-MI tissues.

The pro- or antiangiogenic role of the ANG type 1 and type 2 (AT1 and AT2) receptors in angiogenesis remains controversial (25). Previously we showed that losartan, an AT1 receptor antagonist, significantly improves perfusion and reduces tissue hypoxia (56). To determine whether our mathematical model can successfully predict improvements in tissue oxygenation by various pharmacological interventions, the vascular geometrical and quantitative analysis of hypoxia were performed. The results are shown in Fig. 3. The predicted and experimentally measured hypoxia levels in 1- to 4-wk post-MI tissue. All simulations were carried out with parameters listed in Table 1. All intensity values representing hypoxia levels are expressed as the ratio of intensities in the infarcted over the normal regions.
etry obtained from losartan-treated MI rats (56) was used to simulate the resulting changes in tissue oxygenation. As shown in Fig. 4, our model successfully predicted the changes in oxygenation induced by losartan treatment in infarcted tissue.

Next, the significance of the spatial distribution of microvessels in tissue oxygenation was evaluated by using simulated geometries derived from experimentally obtained normal heart microvascular networks by randomly eliminating capillaries to match the vascular density at different time points after MI. As shown in Fig. 5, the predicted results of levels of hypoxia in morphological geometry, as well as simulated geometry, are not significantly different from experimental data. Similarly, we did not observe a significant difference in hypoxia levels between experimental data and those in simulated networks that were obtained by systematically eliminating vessels from a normal vascular network (data not shown). The results of this study indicated that oxygen distribution patterns in these simulated networks were not significantly different from experimentally obtained morphological vascular geometries, thus indicating that both vascular density and vascular spatial distribution are the primary factors in determining oxygenation in post-MI tissue.

To further elucidate the relative roles of vascular density and spatial distribution in oxygenation in postinfarct tissue, we explored the possibility of using a simple Krogh cylinder model (46) to simulate oxygenation in post-MI tissue. In this Krogh cylinder model vessel diameter was assumed to be the average of all perfused vessels (e.g., 10 μm for 2 wk after MI), and the tissue cylinder area was assumed to be the average tissue area per perfused vessel (e.g., 962 μm² for 2 wk after MI). Oxygen consumption rate throughout the tissue cylinder was assumed to be uniform, and oxygen diffusion rate was assumed to be determined only by the oxygen concentration gradient (see Table 1 for numerical values). The results of this study indicate that, compared with the Krogh cylinder model, our model can more realistically simulate the heterogeneity of tissue oxygenation resulting from the irregular spacing of the microvessels. For example, as shown in Fig. 6 and in agreement with the literature (46), the distributions of predicted PO₂ values between the two models are strikingly different. While our model provides a more realistic distribution of PO₂ values, the Krogh model results in an unrealistically right-shifted distribution with a large fraction of the tissue having a PO₂ close to that of the outer boundary. This study also indicates that vessel distribution can play a significant role in determining oxygenation in the infarct tissue if vessels are evenly spaced.

DISCUSSION

We have developed, and experimentally validated, a mathematical model to study oxygen transport from a capillary network in both MI and normal areas of infarcted heart. This model can be used to reliably simulate distributions of oxygen concentration in infarct rat hearts 1–4 wk after MI and to
simulate the changes induced by proangiogenic interventions such as losartan treatment. Our mathematical model provides detailed information on oxygen distribution in MI and control tissue that is consistent with experimentally obtained data. The experimentally obtained hypoxia levels in post-MI tissues can also be predicted with our simulated geometries, which are based on a random removal of certain number of vessels to match the vascular density of post-MI tissues. These results indicate that both vascular density and the spatial distribution of vessels can be important determinants of oxygenation in the MI tissue. Obviously, vessel distribution plays a more dominant role where the vessels are distributed in “clusters” (e.g., the oversimplified geometry illustrated by the Krogh cylinder model). However, experimentally, aside from the necrotic area, we have not observed tissue areas with large “clustered” gaps in capillary density (56).

Several major strategies have been suggested for rebuilding vasculature in infarcted heart (57), and we recently developed (43) a novel methodology for preferentially delivering drug carriers to post-MI tissue by using upregulated vascular adhesion molecules as targets. However, as explained above, mathematical modeling provides the only realistic tool for determining the distribution and density of the vessels that are needed to support the newly formed cardiomyocytes. We are currently using the mathematical model developed in this study as a tool in guiding the rebuilding of myocardium in the infarcted heart. In its current form this model is not intended to direct clinical treatment (dose of drug, etc.) but rather to predict potential outcomes in an experimental model. The long-term goal of our studies, however, is to develop a model that can be used to predict clinical outcomes after various interventions; we believe such a model may be feasible, especially considering the recent development of new noninvasive methodologies to measure hypoxia levels clinically with probes such as EF5 (59).

In addition to the level of angiogenesis required, time point and duration of treatment are important parameters for an optimized treatment schedule that can be derived from our model. Results from our experimental studies indicated that the best time to rebuild myocardium may be 7–14 days after MI (56). However, our mathematical model indicates that 2 wk after MI 29% of the myocardium is severely hypoxic \( [P_O_2 < 2 \text{ mmHg} ] \) and that the vascular density of the infarcted tissue should reach 75% of normal tissue vascular density to ensure that no areas of the myocardium are critically hypoxic. In fact, as predicted by our model, we have shown (55) that a smaller increase in vascular density (28% of normal tissue vascular density) resulting from systemic VEGF therapy does not result in any significant improvements in cardiac function. The large increase in vascular density required to eliminate hypoxia may be an important reason why many attempts at rebuilding the myocardium with stem cells have yielded disappointing results (47).

In general, oxygen transport in tissue depends on the microcirculatory structure and its associated hemodynamics (4, 16, 17, 19, 20, 36, 44, 45). To build a more anatomically realistic mathematical model of oxygen transport to tissue it may be necessary to build a detailed representation of the three-dimensional microscopic structures of tissue vasculature (3) and to describe the effects of cellular structures, such as cell membrane and organelles, on oxygenation (37). Such models have provided more detailed information on the oxygen distribution in skeletal muscle and other tissues (16, 17, 37, 44). However, these complex three-dimensional models require a large number of parameters that are not all available from experimental data in the case of infarcted myocardium, making model optimization and model behavior studies very difficult (3, 17). Therefore, based on currently available parameters in infarcted tissue, we have developed an efficient model with few parameters and few ad hoc assumptions (assumptions that are not, or cannot be, validated against experimental data currently available in the literature) that is very useful for studying the relationship between changes in vascular geometry and tissue oxygenation.

A current limitation of our model may be the assumption that the oxygen diffusion and/or consumption rates for different areas of tissue are the same, while the in vivo situation in the heart may resemble that seen in other organs such as the brain, where the oxygen consumption rate decreases after hypoxia (29). However, to our knowledge, oxygen consumption in various areas of the infarcted region has not been measured experimentally. Therefore, to limit the number of ad hoc assumptions in our model we have used uniform oxygen consumption throughout the tissue. Nevertheless, our model could account for differential oxygen consumption, as well as hematocrit/hemoglobin concentration, in various areas of post-MI cardiac tissue if those parameters were to become available. The necrotic portion of the scar tissue (mainly composed of fibrillar collagen) is not very metabolically active and as such does not consume significant amounts of oxygen as shown in our previous experimental study (56); hence in our model we have assumed zero consumption in the necrotic region. To improve the accuracy of this model, we are currently developing methodologies to experimentally measure oxygen diffusion and/or consumption rates under different levels of hypoxia and in various areas of cardiac tissue. Another limitation may be that our model does not include all the structural and functional components of the tissue (e.g., heterogeneous distribution of mitochondria within myocytes). However, given the very limited availability of experimental data on the oxygenation and perfusion status of postinfarct tissue, we believe that inclusion of this additional level of detail will result in a mathematical model that cannot be validated experimentally, and hence will be less useful to experimental scientists. Nevertheless, our model is flexible enough that these additional details can be included when the necessary experimental data for validating them become available.

In Figs. 3–5 data are expressed as the ratio of EF5 intensity in the infarct region over that of normal myocardium. While the calculated intensities are a linear function of oxygen concentrations for simulated data, the experimentally obtained EF5 intensity values may not be a linear function of oxygen concentration (23). However, at this time the nature of the relationship between EF5 intensity and oxygen concentration in the myocardium cannot be verified because the calibration of EF5 intensity with oxygen concentration is only available for tumor cells (23). Nevertheless, if this calibration is used to express our simulated data in terms of intensity values and recalculate the intensity ratios, the overall trend of increasing levels of hypoxia over time remains intact. However, in Fig. 3, for example, at 1 and 2 wk after MI the simulated data fit better...
with the experimental data, but the fit between simulated and experimental data at 3 and 4 wk after MI are poorer than those shown in Fig. 3.

In general, a cross section through a tissue overestimates the distance from a point in the tissue to the nearest vessel because not all vessels may be vertical to the cross-sectional plane. If used as a basis for simulating oxygen diffusion, this 2D approach may result in underestimation of tissue oxygen levels. In our studies we have used coronal sections of the heart in which most of the vessels (>80%) are oriented within 30° of vertical (54). These conditions result in a maximum overestimation of 6% in the distance to the nearestperfused vessel (41).

In conclusion, utilizing microvascular morphology of cardiac tissue based on available morphometric images, our model can be used to predict distributions of oxygen concentration in normal and infarcted rat hearts, as well as in infarcted tissue treated with proangiogenic compounds such as losartan. From the minimum oxygen concentration myocardies need to maintain their normal function, we can calculate the number of new perfused vessels needed in the heart to avoid tissue hypoxia, guiding our work of rebuilding vascular networks and myocardium.

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REFERENCES


