Experimental Determination of Diffusion Parameters in Rat Tumor Tissue Using Fluorescent Visualization Methods

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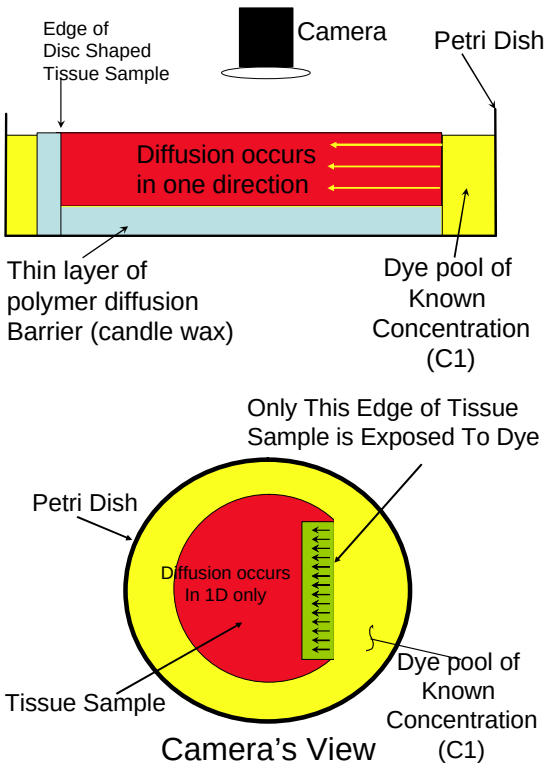
Extended Abstract

The objective of this study is to estimate the diffusion coefficient in solid rat tumor tissue. The transport of effective doses of anti-cancer tumor drugs to solid tumors is very challenging due to the barriers imposed by the tumor and its vasculature [1]. Furthermore, drug metabolizability, the body's natural removal process, and binding to non-target cells [2] make complete utilization of the drug impossible. Drug transport within the tumor is largely dependant upon diffusion rather than convection [1]. The work of Netti et al. [3] and Pluen et al. [4] for example, have shown that the diffusion of large molecules is significantly hindered in tumors with a high collagen content. It is believed the delivery of drugs to the tumor tissue is directly dependent upon the local value of the diffusion coefficient for a particular drug, therefore, being able to predict the distribution of the drug within the tumor tissue region will enable the design of effective anti-cancer drugs that are more readily taken up by the tumor.

The solid tumors used in this study are grown and harvested from rat models. Rats are injected with C-106 and C-108 tumor cell culture lines under the skin in the hip region. The tumors, harvested after 6 weeks, range in sizes from 2 to 5 cm in diameter. Tissue core specimens in cylindrical shapes of 1 cm in diameter, with thicknesses of 1-4 mm and length of 2 to 5 cm are prepared and frozen for later use. The specimens are encased in *wax* (material with a melting point of 50^oC) so that the diffusion direction is restricted. Figure 1 is an illustration of this. Observe that the specimen is viewed from the top by a CCD (charge-coupled device) camera fitted with specially designed light filters. Figure 2 is a an image of one specimen in the dye pool.

Each specimen is first warmed to 38^oC (normal body temperature) and then submerged in a dye solution (AK Fluor or Fluorescein, Akorn Inc, Decatur, Ill.) of known concentration that is also at 38^oC. Most of the specimen will be submerged in the dye solution with only 0.5-1 mm of the specimen not in the dye solution. Images (taken from the side of the specimen that is not touching the dye) of the dye diffusing from the dye pool into the tissue are recorded. A digital frame grabber is used to discretize and store the images. The camera lens has an adjustable field of view from 12 to 150 mm. The images are sampled at 2 frames per second over the duration of each experiment (approximately 30 minutes).

The images are analyzed using a custom LabVIEW©(National Instrument) program developed to record and analyze the pixel intensity and locations over time. Concentration analysis is carried out using linear absorption theory [5] to calibrate the relative solute concentration at the pixel scale to a digitized gray-level value of the recorded images. With



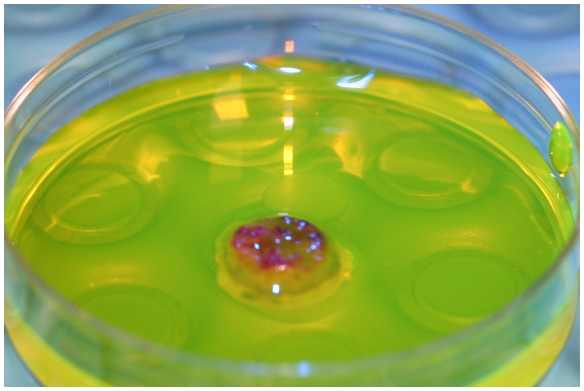
A: The Side View - An illustration representing the proposed experimental setup. The barrier on the bottom and sides of the specimen limits diffusion to one direction so that a one dimensional calculation can be performed for the analysis of the dye front as it moves to the center of the disc.

B: Camera's View from above specimen - One side of the wax is cut to expose tissue to liquid dye.

Figure 1: Measuring the diffusion rate of the dye into the tissue specimen.

the concentration data and the application of Fick's second law, it is possible to calculate local diffusion coefficient values. This calculation is repeated over small, finite line segments, resulting ultimately in knowledge of the spatial distribution of the diffusion coefficients within the tumor. Finally, the experimental diffusion coefficient values will be used in a constitutive model [6] to predict the effectiveness of different drugs as a function of treatment duration, drug concentration, and other relevant parameters.

In summary, this study provides an experimental technique to estimate the diffusion coefficient in solid tumors. The work underscores the importance of understanding the mechanisms of drug transport to address the effectiveness of anti-cancer tumor drugs.



Researcher's Actual View - Disc shaped specimens are placed in a pool of dye of known concentration. The bottom and sides of the disk are coated with wax. Part of the wax is removed from one side to expose tissue to dye.

Figure 2: Image of the tissue specimen in the dye solution.

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