ABSTRACT

Elevated levels of nitric oxide (NO) \textit{in vivo} are associated with a variety of cellular modifications thought to be mutagenic or carcinogenic. These processes are likely mediated by reactive nitrogen species (RNS) such as nitrogen dioxide (NO$_2$) and peroxynitrite formed from the respective reactions of NO with oxygen and superoxide anion. Controlled delivery of these RNS at levels expected to occur \textit{in vivo} is desirable in studying these processes and their role in the etiology of various diseases. Two delivery systems were developed that provide novel capabilities for steady, quantitative exposure of biological targets to RNS over periods from hours to days. Quantitative models are presented that accurately describe the behavior of both systems. The first system achieves NO concentrations of 0.6-3.0 $\mu$M in a stirred, liquid-filled vessel by diffusion from a gas stream through a porous poly(tetrafluoroethylene) membrane. Oxygen, consumed by reaction with NO or by other processes, is supplied by diffusion from a separate gas stream through a loop of poly(dimethylsiloxane) tubing. The adventitious chemistry observed in a prior device for NO delivery [Wang C. \textit{Ann Biomed Eng} (2003) \textbf{31}:65-79] is eliminated in the present design, as evidenced by the close match to model predictions of the accumulation rate of nitrite, the stable end product of NO oxidation. The second system delivers NO$_2$ by direct contacting of a stirred liquid with an NO$_2$-containing gas mixture. Accumulation rates of products in the presence and absence of the NO$_2$-reactive substrate 2,2$'$-azino-bis(3-ethylbenzothiazoline-6-sulfonate) matched model predictions within 15\% for all conditions studied. The predicted steady NO$_2$ concentration in the liquid is on the order of 400 pM, similar to what is expected to be present in extracellular fluids in the presence of 1 $\mu$M NO. This system appears to be the first reported with the capability for sustained, quantitative NO$_2$ delivery to suspended cell cultures. Results from initial efforts to test a novel mixing model for bolus delivery of peroxynitrite to agitated solutions imply that the proposed model might accurately describe mixing in bolus delivery experiments with agitation by vortex mixing, but further work is required to validate the model.