ELECTROCHEMICAL DETERMINATION OF DIFFUSION COEFFICIENTS: OBSERVATIONS AT TISSUE MATRIX BARRIERS AND MAJOR ANOMOLIES IN CONSTRAINED CHANNELS.

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Ultimately, cells are microscale bioreactors and as such respond to the flux solutes, ie to diffusion, albeit driven by concentration gradients. To simplify diffusion measurements, we have established an electrochemical measurement platform enabling rapid, precision measurement of diffusion coefficients. We have used this approach to determine microsolute transport across cultured blood vessel cells[1] and polymeric barriers [2]. In our recent study determined diffusion coefficients at crosslinked collagen I, IV and negatively charged polysugars to serve as simple models for the extracellular tissue matrix. The barriers presented by the various membrane form matrices correlated inversely with protein content, were influenced by the inclusion of charged polymer and were greater for collagen IV *vs* I, this may have biological significance. Four diffusants: H_2O_2, catechol, acetaminophen and ascorbate, were used. Diffusion testing in cartilage and tendon showed there was a major reduction in diffusion coefficients as compared with values in water, and of substantial relevance to cell viability given that these tissues have either little (tendon) or no (cartilage) direct blood supply. Diffusive coefficients in these tissues were 1.09×10^{-8} cm^2s^{-1} - 8.33×10^{-6} cm^2s^{-1}.

Solute permeability was also determined through polymeric track etched membranes. Such membranes have discrete near-cylindrical holes that traverse the full length of the membrane with minimal angular deviation. Their surface pore structure allows ready determination of porosity without the uncertainty tortuosity, typical of many separation membranes - the tortuosity factor. They have served, also, as barrier materials for enzyme based biosensors and are in common use for dual compartment tissue culture where solute exchange is required between the two compartments. Remarkably, no direct measurement of diffusion coefficients have been made at these membranes. The assumption is that solute mass transport, at least, for molecules well below pore diameter must be equivalent to that in bulk water. The assumption here is that any wall effects within the pores must be limited to sub-nm thickness. Our study established, on the contrary, that effective solute diffusion coefficients for the above probe molecules were well down, approximately to 0.01-1% of expected values. This is despite pore diameters of 0.4 and 8.0μm. Remarkably, diffusion retardation was independent of pore diameter, strongly affected by the nature of the polymer (polycarbonate or polyethylene terephthalate) and showed discrimination based on both molecular weight and solute charge. Confirmation of this data was provided by the use of standard cyclic voltammetry. Overall, diffusion coefficients were between 1.43×10^{-10} - 3.17×10^{-7} cm^2 s^{-1}. The possibility has to be considered that intra-pore water structure is different from that in the bulk, and that the material surface is a determinant of this. Further structural and diffusive resistance analysis is warranted to help understand micropore properties and solution behaviour in constrained environments.

1 Zorlutuna P., et al Acta Biomateriala 5, 2451, 2009.

2.Rong Z., Electroanalysis 18, 1703, 2006