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Numerical Modeling of Electroosmotic Push-Pull Perfusion and Assessment of its Application to Quantitative Determination of Enzymatic Activity in the Extracellular Space of Mammalian Tissue

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Abstract

Many sampling methods have been developed to measure the extracellular concentrations of solutes in the extracellular space of mammalian tissue. However, few have been used to quantitatively study the various processes, such as enzymatic degradation, that determines the fate of these solutes. For a method to be useful in this pursuit, it must be able to 1) perfuse tissue and collect the perfusate for quantitative analysis of the solutes introduced and reaction products produced, 2) control the average residence time of the active solutes, and 3) have the appropriate spatial resolution for the process of interest. Our lab previously developed a perfusion technique based on electroosmosis (EO), called EO push-pull perfusion (EOPPP), that is in principle suitable to meet these needs. However, much like the case for other sampling methods that came before, there are parameters that are needed for quantitative interpretation of data but that cannot be measured easily (or at all). In this paper, we present a robust finite element model that provides a deep understanding of fluid dynamics and mass transport in the EOPPP method, assesses the general applicability of EOPPP to studying enzyme activity in the ECS, and grants a simple approach to data treatment and interpretation to obtain, for example, $V_{\max}$ and K_m for an enzymatic reaction in the extracellular space of the tissue. This model is a valuable tool in optimizing and planning experiments without the need for costly experiments.

TOC image

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Supporting Information. Boundary conditions and general fluid and solute properties for FEM simulations, geometry and meshing for FEM models, experimental section for model validation, effect of different parameters on flow rate, effect of molecular weight and charge on species collection by EOPPP, steady-state calculations, average concentration and $Pé$ in the tissue at various X and Z coordinates, effect of current and sampling i.d. on collection efficiency, residence time calculations, comparison of EO- and P-driven flow, detailed discussion of the effect of formation factor on collection efficiency and the need for an internal standard, and correlation plots of inferred enzyme kinetics and actual enzyme kinetics in the tissue ECS.

INTRODUCTION

Techniques for measuring extracellular concentrations of solutes (such as neurotransmitters, metabolites, peptides, drugs) have been around for over half a century. Modern sampling tools, such as microdialysis^{1–8}, low-flow push-pull perfusion^{9–13}, and solid-phase microextraction^{14–16} are valuable assets for answering biological questions based at least in part on analytical measurements of solute concentrations in the extracellular space (ECS)¹⁷. These sampling procedures are a key component of the analytical methods that use them, and thus their influence on the analytical result must be well understood. For this reason, mathematical models of fluid and mass transport in these procedures have been developed and used to refine the application of sampling techniques. Various mathematical models have been developed for microdialysis (reviewed by Kehr)¹⁸. Bungay et al.⁷, following up on earlier and simpler models^{19,20}, created a unifying approach by including both axial and radial diffusion to the probe. The numerical model provides outflow concentration given the inflow and external medium concentrations as well as the probe membrane permeability and geometry. In recent years, Diczfalussy et al.^{21,22} developed a finite element method (FEM) model to predict the volume of tissue accessed by microdialysis. They introduced diffusion tensors from patient magnetic resonance imaging data into the FEM model for accurate computation of patient-specific sampling volume around the microdialysis probe²². This accounts for anisotropy and heterogeneity in the brain, which are important to solute transport in the ECS as shown by Syková and Nicholson^{23,24}. In contrast, there are fewer mathematical models of low-flow push-pull perfusion and solid-phase microextraction. Cepeda et al.²⁵ related sampling-induced cell death from low-flow push-pull perfusion to sampled fluid velocity, local pressure, and shear stress near the sampled site. Alam et al.²⁶ developed a numerical model for solid-phase microextraction that simulates solute partitioning under various conditions of fluid agitation, fiber coating thickness, and the presence of a binding matrix. Taken together, these computational results provide insight into the complex processes that occur during sampling and a means to optimize experimental conditions without the need to perform a large number of experiments.

There are in principle significant advantages to the use of electroosmotic flow to perfuse tissue as long as the tissue itself has a sufficiently large zeta potential, which is true for the brain²⁷. The current path guides the direction of fluid flow and the current magnitude can in principle easily control the flow rate. Thus, we envisioned electroosmotic push-pull perfusion as a novel and useful approach, particularly to measure quantitatively (how fast) and qualitatively (what products) the rates of enzymatic hydrolysis of peptides by

membrane-bound, extracellular peptidases. The first-generation electroosmotic sampling^{28–31} was used to determine extracellular cysteamine in organotypic hippocampal cultures and to characterize enzyme activity in the tissue ECS related to neuropeptide degradation and coenzyme A catabolism. A second-generation^{32,33} electroosmotic push-pull perfusion (EOPPP) technique, which has better spatial resolution than its predecessor, was used to assess differences in product distribution from extracellular peptidase activity acting on the 29-amino acid neuropeptide, galanin, in the CA1 and CA3 regions of organotypic hippocampal slice cultures.

Our long-term goal is to determine quantitative and qualitative differences in enzyme activity over time and in a spatially dependent manner. For a method to be useful in this pursuit, or any investigations of the fate of molecules as they pass through the ECS, it must be able to 1) perfuse tissue and collect the perfusate for quantitative and/or qualitative analysis of the solutes introduced and reaction products produced, 2) control the average residence time of the active solutes, and 3) have the appropriate spatial resolution for the process of interest. EOPPP is in principle suitable to meet these needs. However, much like the case for microdialysis, low-flow push-pull perfusion, and solid-phase microextraction, there are parameters that are needed for quantitative interpretation of data but that cannot be measured easily (or at all) in EOPPP. A robust computational model would be very useful for understanding the fluid and mass transport events, for optimizing conditions, and for use in data interpretation to obtain quantitative results from the perfusion/collection of solutes and their reaction products.

We previously developed a physical model composed of a slice culture and two probes. With this model, we investigated the potential drop in the tissue and correlated that with cell death in order to find “safe” conditions.³¹ We also used the model to show that the flow rate through the tissue culture under an applied current depended on the diameter of the sampling capillary,^{32,33} but this was not discussed quantitatively. The model described in this paper is built upon the previous model and also includes, solute transport and enzyme kinetics. Thus, this model provides a more comprehensive, quantitative understanding of EOPPP, including the factors influencing solute distribution and residence time within the tissue. We show how to use the model to extract kinetic parameters of an enzyme in the tissue ECS based on EOPPP of the perfused substrate and collection of generated product.

THEORY

Finite element numerical model

The model in COMSOL Multiphysics (v5.2) simulates electroosmotic flow (“Electric Currents” and “Free and Porous Media” modules) as well as solute transport and reaction kinetics (“Transport of Diluted Species in Porous Media” module). A 3-D geometry representing the capillary lumens and tissue culture was built on the COMSOL Multiphysics geometry interface using simple shapes and parametric curves (Fig. 1). The buffer droplet between the sampling capillary and tissue surface was represented in the simulation as a truncated cone. The tissue was treated as a rigid, homogeneous porous material. The program applies a current boundary condition to create EO flow.

EO flow in a porous medium^{34–36} can be modeled as a body force resulting in a modified Darcy's law (Eqn. 1),

$$\frac{\eta}{\kappa}u + \nabla P + \rho_{eff}\nabla\phi = 0 \quad [1]$$

where η is the dynamic viscosity (Pa s), κ is the permeability (m²), u is the superficial velocity (m/s), ∇P is the pressure gradient (Pa/m), ρ_{eff} is the effective charge density of the fluid (C/m³), and $\nabla\phi$ is the gradient of the electric potential (V/m). The superficial velocity is the volume flow rate divided by total cross-sectional surface area (including the impermeable portion of the medium). The interstitial velocity is the average velocity in the macroscopic direction of flow through the interstitial space of the porous medium. In order to determine the effective charge density, we started with an equation for superficial electroosmotic velocity in a porous medium^{33,37}:

$$u_{eo} = \frac{\varepsilon_w \zeta \varepsilon}{\eta \lambda^2} \nabla \phi \quad [2]$$

where ε_w is the permittivity of water (F/m), ζ is the zeta-potential (V), ε is the porosity, and

λ is the tortuosity (or $\frac{l}{L}$, where l is the length of the curved path and L is the length of the straight path through the porous medium). By equating the superficial velocities in Eqns. 1 and 2, in the absence of a pressure gradient, the effective charge density is

$$\rho_{eff} = \frac{\varepsilon_w \zeta \varepsilon}{\lambda^2 \kappa} \quad [3]$$

Thus, the equation used for EO-driven fluid flow in the tissue is:

$$\frac{\eta}{\kappa}u + \nabla P - \frac{\varepsilon_w \zeta \varepsilon}{\lambda^2 \kappa} \nabla \phi = 0 \quad [4]$$

In the capillaries and buffer layer between the sampling capillary and tissue, the Navier-Stokes equation is used.

In order to save computational time, we shortened the length of the capillaries from 30 cm to 2 mm. This truncation affects the pressure resulting from differences in ζ -potential between two adjacent media (capillary and tissue) through which the current flows. In order to account for this truncation, we defined boundary conditions at the inlet (to the source capillary) and outlet (from the sampling capillary) as “Hagen-Poiseuille” boundaries that define the pressure drop in the missing length for an incompressible Newtonian fluid with laminar flow:

$$\Delta P = \frac{8\eta L_m}{a^2} \Delta u \quad [5]$$

where P is the pressure drop in the missing length of capillary, L_m is the missing length of the capillary, u is the difference between wall velocity and average fluid velocity at the exit of the truncated capillary, and a is the radius of the capillary. The Hagen-Poiseuille boundary condition takes advantage of the fact that the flow profile is constant in this portion of the capillary to determine the pressure drop that would have been generated if the missing length had been included in the geometry. All boundary conditions are defined on pages S-5 to S-7 of the Supporting Information.

The module “Transport of Diluted Species in Porous Media” uses the following equation for each species, ‘ i ’:

$$\varepsilon \frac{\partial c_i}{\partial t} = R_i - u \cdot \nabla c_i + \nabla (D_{e,i} \nabla c_i) + \varepsilon \nabla (\mu_{ep,i} c_i \nabla \phi) \quad [6]$$

where c_i is the concentration (in M) for solute ‘ i ’ in the interstitial space, $\frac{\partial c}{\partial t}$ (M/s) is the change in concentration with respect to time, R_i is a reaction rate (M/s), u is the superficial velocity (m/s from Eqn. 4), $D_{e,i}$ is the effective diffusion coefficient (m²/s), and $\mu_{ep,i}$ is the effective electrophoretic mobility (m²/Vs). Here, the term “effective” refers to the incorporation of tortuosity to obtain a value of a parameter originally determined in free solution that would be observed macroscopically in the porous medium. The effective

diffusion coefficient in a porous medium is $\frac{D}{\lambda^2}$ ³⁸, where D is the diffusion coefficient in free solution. The terms on the right-hand side of the equation represent, respectively, chemical reaction, convection, diffusion, and electrophoretic migration.

Two approximations were made to simplify the calculations. First, we modeled the brain as a rigid porous medium. Work by Støverud et al.³⁹ demonstrated no significant difference between using a rigid or an elastic model to simulate drug infusion (by pressure) into the brain for tissue permeability greater than 1.82×10^{-15} m². The permeability we used in the model is 10^{-14} m²⁴⁰, thus the rigid model is appropriate for our system. Second, the 25 μ m layer of buffer that exists between the sampling capillary’s end close to the culture and the top surface of the culture is modeled as a fixed truncated cone shape (see Fig. 1). In reality, this is an approximation. The true surface shape depends on the surface tension of the droplet, the pressure outside the droplet, and the pressure within the droplet. The added complexity required to include this effect did not seem justified based on the fact that this droplet is merely a throughway for current and solute to pass from the tissue to the lumen of the sampling capillary.

RESULTS AND DISCUSSION

Model assessment

The FEM model was built based on the assumption that the tissue is a homogeneous porous medium and uses several parameters from the literature, including viscosity ($\eta = 8.9 \times 10^{-4}$ Pa s), zeta potential of the capillaries ($\zeta_{\text{cap}} = -46.5$ mV)¹⁷, zeta potential of the tissue ($\zeta_{\text{tissue}} = -22.8$ mV)⁴¹, peptide diffusion coefficients ($D_{\text{yaGfl}} = 4.45 \times 10^{-10}$ m²/s)⁴², as well as tissue culture porosity ($\epsilon = 0.2$ – 0.6)^{43,44} and tortuosity ($\lambda = 1.2$ – 1.6)^{43,44}. We wanted to know whether results from this model would be closely correlated to experimental observations. Thus, we assessed the transport portion of the model using data for the collection of a D-amino acid peptide ^DY^DAG^DFF^DL (yaGfl) with the EOPPP setup. Due to its D-amino acids, yaGfl is not rapidly hydrolyzed in the tissue and thus makes for a good sampling internal standard (this will be discussed in more detail later when we discuss using the FEM model for simulating enzyme activity). Experimental data for moles of yaGfl collected in ten minutes at two different currents ($i = 5$ and 15 μ A) were normalized to the initial concentration of yaGfl in the source capillary. This ratio, with units of nL, is related to the collection efficiency, the fraction of the moles captured at the sampling capillary to those introduced at the source (Fig. 2). As seen in Fig. 2, this ratio at 5 μ A was 14 ± 2 nL for experiments (mean $\pm$ SEM, $n = 4$) and 22 nL for the model. At 15 μ A, the the ratio was 71 ± 7 nL for experiments ($n = 11$) and 97 nL for the model. Given the complexity of the system, the fact that we are using estimates of tissue properties (e.g. porosity, tortuosity, permeability, etc.), and the general variability that comes with absolute moles collected because of tissue-to-tissue and rat-to-rat differences, the error between experiment and simulations is very reasonable. We also should mention that the currents used in many experiments for measuring enzyme activity are in the 10 – 20 μ A range, so the 15 μ A comparison has more weight.

Fluid transport

There is a linear relationship between the current and electroosmotic flow rate in a homogenous medium³³:

$$U_{EO} = - \frac{\epsilon_w \zeta}{\eta \sigma} i \quad [7]$$

where U_{EO} is the electroosmotic flow rate, σ is the conductivity of the electrolyte itself, and i is the current. However, the ζ -potential of fused silica capillaries, -46 mV¹⁷, is different from that of the tissue, -23 mV⁴¹. For an incompressible fluid in a closed system, EO flow through a medium with non-uniform ζ -potentials results in non-uniform EO velocities. Mass balance requires that pressure be created to augment flow where the magnitude of the EO velocity is lower and to decrease flow where the EO velocity is greater. This generates a pressure gradient in the system^{17,45}. The magnitude of this pressure difference increases linearly with the magnitude of the applied current and decreases nonlinearly with the inner diameter (i.d.) of the sampling capillary (discussed later) for a rigid porous medium of constant temperature containing a fluid with constant viscosity⁴⁵. In EOPPP, we impose a

positive current from the source to the sampling capillary so fluid flows from the source capillary with high EO velocity to the tissue with low EO velocity and then to the sampling capillary with a high EO velocity. Fig. 3A shows a surface plot of the ratio of pressure to current during perfusion at steady state, with red indicating positive pressure and blue indicating negative pressure. Fig. 3B shows a plot of that ratio as a function of distance from the source capillary, along the dashed line indicated in Fig. 3A. The pressure/current ratio drop is around 2 Pa/ μ A at the tip of the source capillary and -0.3 Pa/ μ A at the sampling capillary. This pressure difference, though small, exists across a small distance, so it actually assists EO flow in pumping fluid through the tissue, as the flow rate is higher in the tissue than it would be without this effect.

Our model shows that the contribution of this EO pumping phenomenon to the overall flow rate depends on the size of the sampling capillary⁴⁵ as mentioned above and shown in Fig. 4 (black symbols). The flow rate was calculated by integrating the superficial velocity in the sampling capillary over the cross-sectional surface area. In Fig. 4, it is normalized to the applied current. The magnitude of the EO flow rate/current does not depend on capillary i.d. (blue, Fig. 4). In fact, the FEM calculations agree with Eqn. 7 quantitatively (see blue symbols and black dashed line). (This is further model validation.) To assess the contribution of the pressure generated by the ζ -potential mismatch to the total flow rate, we performed a separate calculation with the generated pressure (shown in Fig. 3) as the only driving force in the absence of current. The pressure-induced flow rate/current ratio calculated for this pressure-only system is shown in red in Fig. 4. We found that for a sampling i.d. of 100 μ m, the pressure-induced flow rate/current ratio is 0.87 nL/min/ μ A, accounting for nearly 60% of the total flow rate (1.63 nL/min/ μ A). On the other hand, for a larger, 250 μ m, sampling i.d., the pressure-induced flow rate/current ratio is 0.09 nL/min/ μ A, which is only about 10% of the total flow rate (0.86 nL/min/ μ A). The contribution of the pressure-induced flow for constant capillary length diminishes at larger sampling i.d.s because generated pressure for an incompressible Newtonian fluid is inversely proportional to the square of the capillary radius, as shown in Eqn. 5. The sampling capillary acts as an electroosmotic pump.

There are several geometric parameters in EOPPP, including source tip i.d., source barrel i.d., how far the pulled tip is inserted into the tissue, sampling i.d., how far the sampling capillary is positioned above the tissue, and the length of the two capillaries (see Fig. S-6 in the SI). Using our model, we found that the majority of the parameters do not significantly affect the flow rate in the ranges we examined (Fig. S-6), with the exception of sampling i.d. (which was discussed in the previous paragraph) and the lengths of the capillaries (see Fig. S-6). The sampling capillary i.d. has more influence on the flow rate than the source capillary i.d. because the source capillary barrel i.d. (200 μ m) is larger than that of the sampling capillary. The lengths of the capillaries affect the pressure generated from the ζ -potential mismatch. The magnitude of the pressure generated is directly proportional to the length of the capillary, as shown by the L_m term in Eqn. 5. This finding is consistent with the fact that in a homogeneous system where the ζ -potential is uniform, the only parameter that affects EO flow is the current, as described by Eqn. 7. This is a favorable outcome. Of the suite of parameters listed above, current (controlling EO flow and contributing to EO pumping), sampling capillary i.d., and capillary lengths (together controlling EO pumping) are the three easiest parameters to control.

An interesting consequence of the EO pumping phenomenon is the ability to sample solutes that are otherwise impossible to obtain via EO alone. For neutral and cationic solutes, electrophoresis does not hinder sampling by EO. (In the case of cations, it actually facilitates sampling.) However, the ability to sample anions is dependent on the relative magnitudes of the bulk fluid velocity and electrophoretic velocity. Under typical experimental conditions (sampling i.d. = 100 μm , current = 10 μA), monovalent anionic solutes with MW equal to or greater than 150 g/mol, such as glutamate and glutathione, can be sampled successfully (see Fig. 5). Fig. S-7 shows that more highly charged anions (e.g. $z = -2$) could be collected if the MW is high enough. If the internally generated pressure is increased further by decreasing sampling i.d., increasing length, or a combination of the two, smaller solutes with the same charge can be sampled. In the example in Fig. 5, we halved the sampling i.d. and doubled the length of the capillaries to 60 cm. As a result, a monovalent anion with a molecular weight of 100 g/mol could be sampled via EOPPP, according to computations (see Fig. 5).

Solute transport

Most of the following discussion is based on the transport of yaGfl through the tissue via EOPPP. We will use the Péclet number in some of the following discussion to understand the relative effects of diffusion and convection. It is defined as

$$\text{Pé} = \frac{\bar{u}b}{\left(\frac{D}{\lambda^2}\right)} \quad [8]$$

where Pé is the dimensionless Péclet number, $\bar{u}$ is the interstitial velocity, and 'b' is the characteristic dimension of the system (in this case, $\sim 100 \mu\text{m}$). Pé can provide a semi-quantitative assessment of the ratio of convective to diffusional solute velocity. When Pé is much less than one diffusion dominates while when Pé is much greater than one convection dominates. In the following, the stated concentrations of the solute in the tissue culture are all based on moles per volume of extracellular space.

Time-dependent calculations of the evolution of the solute distribution profile in tissue for the peptide yaGfl are summarized in Fig. S-8. The radially averaged solute concentration inside the sampling capillary reaches 95% of the steady-state level after 65, 42, and 32 s for $i = 10, 30, \text{ and } 50 \mu\text{A}$, respectively. Fig. 6A shows a set of surface plots of concentration profile at $t = 600 \text{ s}$ for current = 0 (diffusion-only), 10 μA , 30 μA , and 50 μA EOPPP. Interestingly, the concentration profiles are asymmetrical: solute transport is clearly biased toward the sampling capillary, driven by the current flow between the two probes. This demonstrates an important advantage of EOPPP over diffusion-based as well as pressure-driven methods: there is a straightforward, instrumental means (i.e., current source) to establish flow rate with some control over the directionality in the transport in tissue. This aspect is useful for introducing solutes to and sampling from specific regions, which is precisely what is needed to study activities of various processes, such as degradation, that affect the fate of solutes. The plots also reveal that an increase in current results in an increase in the perfused volume in the tissue. We estimated these volumes by determining

the volume of the tissue where $Pé$ is greater than one and convection dominates. These volumes are 7, 33, and 49 nL for 10, 30, and 50 μA , respectively. The shape of these volumes resemble hemispheres in Fig. 6C, so the radii based on these volumes are 150, 250, and 286 μm , respectively. These provide a semi-quantitative estimate of the spatial resolution that can be achieved at different currents. Fig. 6B is a bar graph showing what percentage of the total moles of solute in the tissue at steady-state is either in diffusion-dominant regions ($Pé < 1$) or convection-dominant regions ($Pé > 1$). An increase in current increases the proportion of solutes in the high $Pé$ region, with only about 40% of the solute in convection-dominant regions at low current (10 μA) compared to more than 80% of the solute in the equivalent region at high current (50 μA). This suggests that an increase in current would result in an increase in solute transfer from the source capillary to the sampling capillary. One prominent feature of both Fig. 6A and Fig. 6C is the gradient of concentration that exists in the tissue. The highest concentration in the tissue is adjacent to the source capillary tip, with tissue concentrations that are nearly 100% of the initial concentration in the source capillary (c_0). This concentration decreases to approximately 10–20% of c_0 in the tissue directly under the sampling lumen and roughly 1% near the bottom of the tissue culture. In fact, from the eagle-eye view of the concentration distribution at the surface of the culture (far right in Fig. 6C), it is clear that by the time the solutes reach the tissue surface to be collected into the sampling capillary, there is a gradient of concentration within the convection-dominant zones, the boundaries of which are outlined by the white line. An increase in current increases the area of the tissue surface that is perfused with solutes. An increase in current, however, also increases the area of the tissue where convection dominates. This means that the average concentrations within the regions where $Pé$ is greater than one *decreases* with *increasing* current. This is initially counterintuitive but it makes sense given the concentration profiles and the relative sizes of the convection-dominant zones seen in Fig. 6C. Fig. S-9 in the SI illustrates this further. It shows plots of the average concentration and the average $Pé$ in the area where $Pé$ is greater than one in planes perpendicular to those shown in Fig. 6C. The concentration is low far from the source tip. It is a maximum at the source capillary tip, and then decreases to nearly zero within 100 μm below the tip and within 200 μm laterally. The $Pé$ profile is similar to the concentration profile in both directions, with the highest value of $Pé$ at the source capillary tip. In the context of enzyme activity, this means that the concentrations of the substrates and the enzyme-substrate reaction time vary spatially. We will explore how this affects data interpretation relating to *in vivo* enzyme kinetics measurements in a later section.

We define the collection efficiency of EOPPP as the moles of solute, yaGfl, collected divided by moles of solute injected. We hypothesize that the factors that significantly affect collection efficiency are those that control convection, diffusion, or both. We discussed previously how the sampling capillary i.d. influences the flow rate by affecting the magnitude of the internal pressure gradient generated due to the ζ -potential mismatch. Fig. S-10 shows that an increase in sampling i.d. decreases the collection efficiency. Doubling the sampling i.d. from 100 to 200 μm , for example, reduces the collection efficiency from 44 to 40%. This small change is a result of two competing factors. The larger i.d. column creates a smaller pressure gradient, which reduces collection efficiency, but the collection area is larger which increases collection efficiency. We also discussed previously that applied

current is the other major factor that controls flow rate. Fig. S-10, demonstrates that increasing the applied current from 5 to 55 μA increases the collection efficiency from 33 to 73%. This is consistent with the observation made above that an increase in current results in a higher proportion of solutes in convection-dominant zones.

In addition to the effects of changing experimental parameters on collection efficiency (e.g. sampling i.d. and current), there is an effect of the local tissue environment on collection embodied in ϵ and λ . Because ϵ and λ are interdependent, we examined these two

properties of the porous medium collectively as a single dimensionless ratio, $\left(\frac{\epsilon}{\lambda^2}\right)$, called the formation factor (fF). This is a geological term that relates electrical resistivity in free solution to that in a porous medium⁴⁶. Our calculations show that changes in fF had little effect on EO flow rate itself (see Fig. S-12). However, as seen in Fig. 7, there is a significant effect of fF on solute transport. Increasing the fF decreased collection efficiency at all applied currents. To understand why, we need to look at residence time.

Residence time is the length of time any individual solute spends in the tissue. It is an important parameter especially for enzyme kinetics because it determines how long the solutes, e.g. peptide substrates, are exposed to the different processes, e.g. degradation, in the ECS. The trajectories of the individual solute molecules in EOPPP will vary at least due to convection, diffusion, and the tissue environment. The consequence is that in any experiment there will be a distribution of residence times. We applied a Gaussian solute concentration pulse of 0.05 s standard deviation at the source capillary and observed the solute leaving the tissue over time at the interface between the tissue and the buffer layer. From this time distribution, we determined the first moment, t_R , which is the mean time that the solute molecules spend in the tissue. Values of t_R for fF 0.1, 0.2, and 0.3 are 4.2, 4.8, and 5.3 s, respectively, for applied current of 10 μA (see Fig. S-11). Thus, an increase in fF results in an increase in the average residence time in the tissue. In order to understand why, we need to revisit Péclet.

Fig. 8 demonstrates how the fF affects Pé. On the left, the zones where convection and diffusion dominate are presented graphically in a false color cross sectional surface plot. Brown indicates zones where convection dominates, while the other colors indicate zones where diffusion dominates. On the right, the same information is presented in a more quantitative manner in the form of a line plot. Pé is between 400 (fF = 0.3) and 1400 (fF = 0.1) at the source probe tip and decreases to 5 (fF = 0.3) to 12 (fF = 0.1) at the tissue surface under the sampling capillary. These data suggest that if the tissue is less porous and more tortuous, 1) the volume of the tissue where convection dominates increases in size, and 2) there is a greater ratio of convective to diffusional solute transport in the tissue. For example, the average interstitial velocities in the volume of tissue where Pé is greater than one are 6.7, 5.9, 5.6 $\mu\text{m/s}$ for fF= 0.1, 0.2, and 0.3, respectively ($i = 10 \mu\text{A}$). A decrease in fF results in higher current density in the ECS, thereby causing an increase in the interstitial velocity by EO. In contrast, the effective diffusion coefficient in a porous medium is related to the free diffusion coefficient by $1/\lambda^2$ ³⁸, thus a decrease in fF results in a decrease in effective diffusion coefficient in the tissue. While interstitial velocity is increased by a decrease in fF, diffusional transport is reduced, resulting in higher collection efficiency in

low-porosity/high-tortuosity environments. We note that pressure-driven velocities are heavily dependent on the permeability of the tissue, in contrast to EO-driven velocities, which are only minimally affected. (Rearranging Eqn. 4 and taking the pressure gradient to be zero shows that the superficial electroosmotic velocity is independent of permeability in a homogeneous medium at constant pressure.) This is explored in a simple model calculation in Figs. S-15 and S-16. These figures show that EO flow delivers more fluid and solute to regions with lower fF than those with higher fF . In fact, the greater the difference in porosity and tortuosity between two adjacent regions, the more effective EO flow is at transporting than its pressure counterpart.

Quantitative estimation of enzyme $V_{\max}$ and K_m

We have discussed thus far the fluid dynamics and mass transport of solutes in the tissue during EOPPP, the spatial resolution that can be achieved under various experimental conditions, the ability to control experimental parameters to tune the perfusion rate and reaction time, as well as the effect of local tissue environment on collection. These discussions have provided a broad understanding of the perfusion process and its general applicability to studying reactions in the ECS. This last section addresses how to obtain quantitative information about enzyme activity in the tissue from the perfusion data. An example reaction that can occur in the tissue is as follows:

where YGGFL is Leu-enkephalin, a common endogenous opioid peptide in the brain ECS, and GGFL is the major hydrolysis product after cleavage by membrane-bound aminopeptidase(s) whose catalytic domains face the ECS^{17,28,33}. The following discussion assumes a Michaelis-Menten model but other appropriate models could also be used.

In conventional laboratory *in vitro* measurement of enzyme kinetics, observed rates are so-called “initial rates” because the experimental observation is the reaction rate, and the initial substrate concentration changes very little during the monitoring of the reaction. The Michaelis-Menten, or an analogous rate equation, is directly fitted to the data to derive parameters related to the reaction. In tissue measurements, this is not the case. When using natural substrates, observing the rate in real-time is difficult so a measurement of substrate or product concentration is often taken after a specific reaction time has passed. In addition, the substrate concentration may change significantly as it passes through the tissue. Thus, the integrated form of the Michaelis-Menten equation must be used instead. Furthermore, there is a range of substrate concentrations in the tissue due both to reaction and diffusion (see Fig. 6). Thus, the parameters derived from fitting the integrated Michaelis-Menten equation to the measured quantities may need to take into account the difference between the laboratory initial substrate concentration and the actual initial substrate concentration in the tissue.

Classical Michaelis-Menten kinetics describes the rate of product formation as a function of $V_{\max}$, the limiting rate at saturating substrate concentrations, K_m , the substrate concentration when the observed rate is half of $V_{\max}$, and S_0 . Beal first derived a closed form solution for

product (or substrate) concentration as a function of S_0 and time⁴⁷ (and analytical approximations have been described by Golik⁴⁸):

$$\frac{P}{S_0} = 1 - \frac{K_m}{S_0} \cdot W \left\{ \frac{S_0}{K_m} \cdot \exp \left(\frac{S_0 - V_{\max} t}{K_m} \right) \right\} \quad [9]$$

where P is the concentration of product generated after time ' t ' and W is the Lambert W function^{49,50} that can be found in several commercial software packages (e.g., Matlab, MathCad, Mathematica). An implicit solution for product or substrate can be derived as well^{51,52} which can be solved with a nonlinear solver. In order to extract the appropriate kinetic parameters, P/S_0 , S_0 , and t must be known. The residence time, t , was discussed above. We consider P/S_0 , and S_0 below.

P/S_0 . During a sampling experiment, we can experimentally vary S_0 by changing the concentration of S in the fill solution in the source capillary. After perfusion is complete, we measure the concentration of product P , substrate S , and internal standard IS collected in the sampling capillary by coupling EOPPP with capillary liquid chromatography (with absorbance, fluorescence, or mass spectrometric detection). The internal standard, yaGfl, has properties similar to the substrate. As we make the IS_0 the same as S_0 , experimentally measured substrate and product concentrations are normalized to the measured IS concentration to give estimates of S/S_0 and P/S_0 respectively. This minimizes the effect of differences in fF on collection efficiency as well as analytical errors on the measured quantities (see pages S-20 to S-21 of the SI).

We simulated a simple one-enzyme, one-substrate, one-product system that obeys the Michaelis-Menten model, for which the rate of product generation in the tissue domain is

equal to $\frac{V_{\max} S}{K_m + S}$. The geometry is the same as that described earlier for the EOPPP system. We simulated measurements at thirteen different values of S_0 ranging from 1 μM to 10 mM and nine different pairs of $V_{\max}$ and K_m values (all combinations of $V_{\max} = 1, 10, 50 \mu\text{M/s}$; $K_m = 100, 200, 500 \mu\text{M}$). It is important to note that the initial substrate concentrations used in this simulation span an extremely wide range encompassing pure zero order (the limit of Michaelis Menten at high substrate concentration), pure first order (low substrate concentration) and fractional order. Only in this way can both kinetic parameters, $V_{\max}$ and K_m , be determined.

The simulation provided the P/IS ratios, a surrogate for P/S_0 , in the sampling capillary, for each S_0 after 600 s of perfusion as we do experimentally. The simplest approach to obtain Michaelis Menten parameters is to fit Eqn. 9 to P/S_0 , S_0 , and the first moment of the reaction time distribution t_R to obtain values of $V'_{\max}$ and K'_m . The question is whether or not we can obtain estimates of $V_{\max}$ and K_m (the true values) from these primed values. The *in silico* experiments demonstrates that indeed we can. $V'_{\max}$ and K'_m values derived from the fitting were plotted against actual $V_{\max}$ and K_m values defined in the simulation. The fitting was done using both the entire time distribution and the first moment to compare and contrast the validity of using t_R . These results are summarized in the Fig. 9 and in the SI in

Fig. S-17. $V_{\max}$ and $V'_{\max}$ are directly proportional to each other when using either the full residence time distribution (slope = 10.6 ± 0.3 , $\pm$ SE) or the first moment (slope = 11.3 ± 0.4) and the proportionality is independent of K_m (Fig. 9A). K_m and K'_m are directly proportional as well using either the full time distribution (slope = 2.8 ± 0.2 , Fig. 9B) or the first moment (slope = 3.0 ± 0.6), but only for low values of $V_{\max}$ ($< 10 \mu\text{M/s}$). When $V_{\max}$ is high (i.e. $> 10 \mu\text{M/s}$), the full regression equation for K'_m vs K_m with a nonzero intercept should be used, taking into account that the magnitude of the intercept is $V_{\max}$ -dependent (see Fig. S-17 in the SI). Finally, the kinetic parameters estimated from fitting either P/S_0 or S/S_0 are not significantly different from each other for this single enzyme system that converts S to P (see Fig. S-17). Altogether, this provides a simple but reliable approach for data treatment and interpretation without the need for precise knowledge of S_0 in the ECS.

CONCLUSION

Using FEM calculations, we have demonstrated the suitability of EOPPP as a method for measuring enzyme activity that can equally well be applied to other processes that affect the fate of solutes in the ECS. EOPPP can be used to introduce exogenous solutes via the source capillary and collect both exogenous and endogenous solutes (as well as any reaction products) via the sampling capillary. There are three instrumental parameters, namely current, sampling i.d., and capillary lengths that control the perfusion rate, the Péclet number, and, consequently, the residence time of the solutes in the ECS. Thus, the experimenter has control over the reaction time between the solutes and the enzymes in tissue. The current also influences the spatial resolution of the measurement.

In addition to the three instrumental parameters, we found that the tissue's local geometry (formation factor) also significantly affects Pé and collection efficiency. Our simulations demonstrate that by using an internal standard that has similar diffusion coefficient as the solute of interest and that cannot be degraded by enzymes minimizes the effect of tissue properties on collection efficiency. All quantitative measurements of the amount of solute collected should thus be reported as a ratio to the amount of internal standard collected. Moreover, by making the initial substrate and internal standard concentrations equal, the amount of internal standard collected can be used as a surrogate for the amount of substrate collected if no enzymes had been present.

There is a spatial distribution of solute concentrations and velocities. Both quantities follow a similar profile, with maximum levels near the source capillary tip that decreases steadily toward the tissue surface near the sampling capillary. In the context of enzyme kinetics, this means that kinetic parameters derived from the data must account for these variations. *In silico* experiments using the Michaelis-Menten model demonstrate that useful information about $V_{\max}$ and K_m can be obtained despite the complex profiles of S_0 and Pé in the ECS. In applying EOPPP to biological questions, there are generally “experiments” and “controls.” The ratios of kinetic parameters (experiment to control) will be much less affected by the distribution of solute concentration and velocity in the ECS.

We conclude that EOPPP is generally applicable to studying enzyme activity in the ECS. The spatial resolution is on the order of 100 - 200 μm . Conditions can be altered to improve

collection of low-molecular weight anions. The model grants a simple approach to data treatment and interpretation to obtain useful information about actual kinetics in the tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(Left) Side-view schematic showing the general setup of EOPPP, in which a source capillary is inserted into the tissue at a controllable depth and the sampling capillary rests on top of the tissue at some controllable depth. A buffer layer on top of the tissue maintains electrical contact between the sampling capillary and the tissue. Application of a current drives fluid and mass flow from source to the tissue to the sampling capillary. Not drawn to scale.

(Right) The same geometry built in COMSOL Multiphysics showing the different experimental parameters. Drawn to scale. The walls of the capillaries are not shown.

Figure 2.

Plot of moles of yaGfl collected divided by initial yaGfl concentration in the source capillary [yaGfl]₀ at the two different currents 5 and 15 μA .

Figure 3.

A) False color surface plot of pressure (normalized to applied current) in the source capillary, tissue and sampling capillary. The plane goes through the symmetry axis of the capillaries. B) Pressure/current vs. distance from the source tip (at the source center) along the indicated dashed line. While there is pressure drop in the capillaries, most of the pressure drop occurs between the two capillary tips in the tissue. The sampling capillary i.d. is 100 μm.

Figure 4.

Plot of flow rate/current ratio as a function of sampling i.d. (black). Contribution from EO flow is in blue. Contribution from pressure (P) is in red. The total flow rates are shown in black (EO + P). Dashed line indicates the current-normalized EO flow rate predicted by Eqn. 7.

Figure 5.

False color surface plot of concentration at $t = 100$ s for various conditions. On the left, a series of surface plots show the concentration gradients for increasing molecular weight (from left to right) and increasing sampling capillary i.d. (from bottom to top). Molecular weights are 50, 100, and 150 g/mol going from left to right. Dimensions for the top three are as follows: source capillary 200 μm (barrel) 20 μm (tip), sampling capillary 100 μm , length of capillaries were 30 cm each. Dimensions of the bottom three series are as follows: source capillary 100 μm (barrel), 10 μm (tip), sampling capillary 50 μm , and length of capillaries were 60 cm each. On the right, the concentration gradient for sampling of a neutral solutes ($z = 0$) is shown as a control.

Figure 6.

A) False color surface plot of the concentration profile at $t = 600$ s for $i = 0$ (diffusion only), 10, 30, and 50 μA . All concentrations are expressed as a percentage of initial concentration in the source capillary, c_0 . B) Distribution of solute in the tissue culture between regions with $Pé$ greater than one (red) and less than one (blue). C) 3D plot of concentration in several parallel XY planes at different Z positions. The concentration at the tissue surface is shown in more detail (from an eagle-eye perspective) on the right in order to illustrate the concentration gradient as well as the boundaries (white line) where $Pé = 1$ for the three different currents. The brownish-red ellipse/white ring left of center is the source capillary lumen.

Figure 7. Plot of collection efficiency as a function of formation factors under three different currents (10, 30, and 50 μ A). Collection efficiency was calculated as moles of yaGfl collected divided by moles of yaGfl injected. Shaded region indicate the range of values for fF for healthy organotypic hippocampal slice cultures.

Figure 8.

(Left) Series of false color surface plots showing the effect of fF on $Pé$. (Right) Line plot of $Pé$ as a function of distance from source tip along the white dashed line shown in left panel ($fF = 0.1$). Distances 0–100 μm are the tissue region, whereas 100–150 μm is the buffer layer between the sampling capillary and tissue. $i = 10 \mu A$ for all plots here. Analogous plots for $i = 30$ and $50 \mu A$ are shown in Fig. S-13 in the SI.

Figure 9.

Correlation plot of inferred values of $V'_{\max}$ and K'_m vs. actual values of $V_{\max}$ and K_m in the tissue ECS. A) There was no statistically significant difference in using the time distribution or the first moment to estimate $V'_{\max}$ and the values of $V'_{\max}$ were not K_m dependent. The values of $V'_{\max}$ and $V_{\max}$ are directly proportional. B) Correlation plot of K'_m vs. K_m when the time distribution is used. For $V_{\max} = 10 \mu\text{M/s}$, K'_m and K_m are directly proportional. For high $V_{\max}$ ($>10 \mu\text{M/s}$), the nonzero intercept is statistically different from zero.