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Modeling competitive cytokine adsorption dynamics within hemoadsorption beads used to treat sepsis

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Abstract

Extracorporeal blood purification is a promising therapeutic modality for sepsis, a potentially fatal, dysfunctional immunologic state caused by infection. Removal of inflammatory mediators such as cytokines from the blood may help attenuate hyper-inflammatory signaling during sepsis and improve patient outcomes. We are developing a hemoadsorption device to remove cytokines from the circulating blood using biocompatible, porous sorbent beads. In this work, we investigated whether competitive adsorption of serum solutes affects cytokine removal dynamics within the hemoadsorption beads. Confocal laser scanning microscopy (CLSM) was used to quantify intraparticle adsorption profiles of fluorescently labeled IL-6 in horse serum, and results were compared to predictions of a two component competitive adsorption model. Supraphysiologic IL-6 concentrations were necessary to obtain adequate CLSM signal, therefore unknown model parameters were fit to CLSM data at high IL-6 concentrations, and the fitted model was used to simulate cytokine adsorption behavior at physiologically relevant levels which were below the microscopy detection threshold. CLSM intraparticle IL-6 adsorption profiles agreed with predictions of the competitive adsorption model, indicating displacement of cytokine by high affinity serum solutes. However, competitive adsorption effects were predicted using the model to be negligible at physiologic cytokine concentrations associated with hemoadsorption therapy.

Keywords

competitive adsorption; blood purification; CLSM; sepsis; cytokine

1. INTRODUCTION

Sepsis is a systemic inflammatory state caused by infection, and is characterized by over-production of cytokines in the bloodstream and tissues. Removal of both pro- and anti-inflammatory cytokines from the circulating blood may attenuate the dysfunctional inflammatory response and improve patient outcomes in the setting of severe sepsis [1-2]. We are developing an extracorporeal hemoadsorption device to remove cytokines from the blood using biocompatible, porous polymeric beads. Cytokine removal within the device is mediated by diffusion into the sorbent pores, and physical adsorption to the polymeric surface via hydrophobic interactions. Essential large molecular weight molecules such as

albumin and immunoglobulins are restricted from the sorbent interior through pore size exclusion, thereby minimizing removal of these constituents from the blood. A biocompatible coating on the sorbent surface permits direct perfusion of whole blood through the device, eliminating the need to separate cells from the plasma. Hemoadsorption therapy using our device has demonstrated improved survival in a rat sepsis model [2-3], and may serve as a novel adjuvant therapy for the treatment of severe sepsis.

Many authors have investigated protein transport within sorbent materials, typically utilizing commercial chromatography sorbents (ion exchange, affinity, hydrophobic interaction, etc.) and simple, well characterized protein solutions [4-7]. While these techniques offer insight for optimization and scale-up for certain industrial applications, they are not suitable for analysis of complex feed solutions such as whole blood or plasma used in a hemoadsorption device [8]. Recent developments in sorbent-based blood purification modalities indicate a need for further understanding of mass transport within these materials [9-11]. Specifically, clinically relevant analyses are necessary to characterize mass transport under physiologic conditions, a criterion that is not addressed in traditional chromatography studies where parameters such as pH, protein concentration, and ionic strength can be manipulated.

Our group previously developed a simple mathematical model to characterize cytokine transport in hemoadsorption beads [12], and within devices fabricated from these beads [13-14]. Our original model analysis assumed that competitive adsorption of serum solutes would have negligible effects on cytokine removal dynamics under physiologic conditions [13]. The current work directly tests this hypothesis by examining cytokine adsorption dynamics within single sorbent beads in serum. Multicomponent protein adsorption has been investigated in various types of chromatography sorbents by others [8, 15-20]. Martin, *et al.* [20] developed a two component pore diffusion model to predict intraparticle concentration profiles in ion exchange SP Sepharose FF beads, and validated the model using batch and shallow-bed adsorption experiments. Gallant [15] described multicomponent adsorption in ion exchange particles using the steric mass action model (SMA) [21], and observed displacement of low affinity protein and salt components by a higher affinity protein. Bak and colleagues [8] utilized a complex feedstock (rabbit antiserum) to investigate removal of antibodies by various affinity-based sorbents. They developed a lumped parameter model and quantified Langmuir kinetics through equilibrium and batch adsorption studies.

In this study, confocal laser scanning microscopy (CLSM) was used to quantify intraparticle adsorption dynamics of fluorescently labeled IL-6 in serum, and results were compared to predictions of a competitive adsorption model. CLSM is a powerful technique for visualizing solute transport within single sorbent particles, and has been used extensively to study transport phenomena within various types of sorbent materials [22-28]. We present for the first time a study of intraparticle mass transport using CLSM in whole serum. Quantifying cytokine adsorption dynamics within the hemoadsorption beads is technically challenging due to (1) low cytokine concentrations typically found in sepsis ($< 1\text{ ng/ml}$), and (2) difficulties of performing CLSM in whole serum due to autofluorescence of adsorbed serum solutes. In this work, we used higher IL-6 concentrations necessary to achieve adequate CLSM signal to noise ratios, and fit a multicomponent model to the observed IL-6 intraparticle intensity profiles. The fitted model was then used to simulate physiologically relevant IL-6 concentrations that were under the detection limit of the CLSM technique. Results indicate that a two component model can predict competitive adsorption phenomena between cytokine and serum components within the sorbent beads, and that these competitive effects are likely negligible at physiologic cytokine levels present during hemoadsorption, as assumed in our original model analysis work.

2. MATERIALS & METHODS

2.1. Materials

Recombinant human IL-6 (MW = 21kD, >95% purity) and an NHS-activated fluorophore (DyLight™ 549, MW = 982Da) were purchased from Thermo Scientific (Rockford, IL). IL-6 was conjugated with the fluorophore as follows: 15µg dried fluorophore was reconstituted in 100µl of 10mM phosphate buffered saline (PBS) and 8µl of 0.67M sodium borate buffer. 50µl reconstituted fluorophore was added to 250µl IL-6 (40µg/ml) in PBS and incubated in the dark for 60min. Unreacted fluorophore was removed using resin spin columns provided by the manufacturer. CytoSorb™ beads were provided by CytoSorbents™, Inc. (Monmouth Junction, NJ). CytoSorb beads are polystyrene-divinylbenzene porous particles (450µm avg. particle diameter, 67% porosity, 1.02g/cm³ density, ~1-5nm nominal pore diameter, 850m²/g surface area) with a biocompatible polyvinyl-pyrrolidone coating.

2.2. Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) was used to quantify intraparticle adsorption profiles within the sorbent beads. Labeled IL-6 was spiked into horse serum (Invitrogen, Camarillo, CA) to achieve cytokine concentrations of ~1µg/ml. 1ml aliquots of IL-6 in horse serum or horse serum alone were incubated with 1.5mg CytoSorb beads for 2.5hr, 5hr, and 21hr on a rocker at ambient temperature. At the specified time points, beads were removed from solution, sliced in half using a thin razor blade, and placed in a droplet of PBS on a cover slip for CLSM analysis. An Olympus FluoView™ FV1000 confocal microscope outfitted with a UPlanSApo 20X/.75 oil objective and a HeNe laser (543nm excitation, 572nm emission) was used for all confocal imaging. During image capture, the microscope objective was focused such that the confocal plane was localized within the bead, close to the sliced edge to minimize signal loss through the bead. Images were acquired by horizontal scan at 1024×1024 pixel resolution, corresponding to 0.621µm pixel size. Digital images of sliced beads were analyzed using ImageJ software (National Institutes of Health), and intraparticle intensity profiles were generated by quantifying a rectangular segment of the image across the diameter of each bead (4 – 5 beads were imaged at each incubation time point). Refer to Kimmel *et al.* [12] for further details regarding the CLSM technique.

Baseline autofluorescence profiles from adsorbed serum solutes at each time point were quantified by averaging CLSM intraparticle intensity values for 4 – 5 beads incubated only with serum. This procedure was repeated using different serum/bead samples, and intensity data was found to be repeatable using the same microscope and laser parameters. Mean baseline serum intensities were subtracted from mean IL-6 intensities at each radii point to separate serum autofluorescence signal from IL-6 fluorophore signal. Resulting intraparticle intensity data (*I*) were normalized by the maximum intensity within each particle (*I*_{max}), and spatial position (*r*) was normalized by the average particle radius (*r*/*R*).

2.3. Theoretical Model Development

Mass transport within the sorbent beads is mediated by diffusion into the porous structure, and physical adsorption to the interior pore walls, putatively due to hydrophobic interactions between the solute and polystyrene-divinylbenzene surface. The governing equation for adsorption/diffusion of species *i* within a single sorbent bead is:

$$\rho \frac{\partial q_i}{\partial t} = D_i \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c_i}{\partial r} \right) \quad (\text{Eq. 1})$$

where $q_i(r,t)$ is the mass density of adsorbed species i per bead mass, at radial position, r , within the bead, D_i is the effective intraparticle diffusion coefficient of species i , ρ is the bead density, and $c_i(r,t)$ is the mass concentration of species i within the liquid phase of the sorbent pores. The following assumptions are made: (1) film diffusion effects are negligible, (2) concentration in the liquid phase of the pores is much smaller than concentration in the adsorbed phase, (3) intraparticle adsorption is fast compared to diffusion, such that local equilibrium applies, (4) adsorption is modeled using the multicomponent Langmuir

isotherm, where $q_i = \frac{q_i^{max} K_i C_i}{1 + \sum_j K_j C_j}$. A nondimensional form of the governing equation is created using:

$$r^* = r/R \quad q_i^* = q_i/q_i^{max} \quad c_i^* = c_i/c_i^{in} \quad t^* = t/t_s$$

where R is the average particle radius, q_i^{max} is the maximum adsorbed mass of species i per bead mass, c_i^{in} is the initial bulk concentration of species i , and t_s is a time scale corresponding to the experimental conditions used in the study. Dropping asterisks and combining parameters:

$$\alpha_i \frac{\partial q_i}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c_i}{\partial r} \right) \quad (\text{Eq. 2})$$

$$\text{where } \alpha_i = \frac{\rho q_i^{max} R^2}{D_i c_i^{in} t_s}.$$

Mass transport of serum components (lumped for simplicity as a single species, a) and cytokine (species b) are modeled in a two component competitive adsorption system:

$$\alpha_a \left(q_{aa} \frac{\partial c_a}{\partial t} + q_{ba} \frac{\partial c_b}{\partial t} \right) = \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c_a}{\partial r} \right) \quad (\text{Eq. 3})$$

$$\alpha_b \left(q_{bb} \frac{\partial c_b}{\partial t} + q_{ab} \frac{\partial c_a}{\partial t} \right) = \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c_b}{\partial r} \right) \quad (\text{Eq. 4})$$

where q_{ij} terms are derived from the two component Langmuir isotherm equation:

$$\begin{aligned} q_{aa} &= \frac{\partial q_a}{\partial c_a} = \frac{K_a + K_b K_b C_b}{(1 + K_a C_a + K_b C_b)^2} & q_{bb} &= \frac{\partial q_b}{\partial c_b} = \frac{K_b + K_b K_a C_a}{(1 + K_a C_a + K_b C_b)^2} \\ q_{ba} &= \frac{\partial q_a}{\partial c_b} = \frac{-K_a K_b C_a}{(1 + K_a C_a + K_b C_b)^2} & q_{ab} &= \frac{\partial q_b}{\partial c_a} = \frac{-K_a K_b C_b}{(1 + K_a C_a + K_b C_b)^2} \end{aligned}$$

Here we define K_i as a dimensionless “relative affinity” coefficient, given by $K_i = c_i^{in}/c_{i50}$, where c_{i50} is the concentration required to saturate 50% of sorbent sites. We can consider cytokine adsorption to be in a “low relative affinity” regime ($K_b \ll 1$) given $c_b^{in} \sim O(10^{-6} \text{ mg/ml})$ and c_{50} for protein adsorption in typical sorbent beads $\sim O(10^{-1} \text{ mg/ml})$ [29]. Hence, cytokine concentrations in our application are much less than those necessary to reach $1/2$

bead saturation (c_{50}). Assuming that competing serum components are at higher bulk concentrations than cytokine ($K_a \gg K_b$), adsorption terms are simplified to:

$$\begin{aligned} q_{aa} &= \frac{\partial q_a}{\partial c_a} = \frac{K_a}{(1+K_a C_a)^2} & q_{bb} &= \frac{\partial q_b}{\partial c_b} = \frac{K_b}{(1+K_a C_a)} \\ q_{ba} &= \frac{\partial q_a}{\partial c_b} = \frac{-K_a K_b C_a}{(1+K_a C_a)^2} & q_{ab} &= \frac{\partial q_b}{\partial c_a} = \frac{-K_a K_b C_b}{(1+K_a C_a)^2} \end{aligned}$$

From these relationships, we can see that q_{aa} is proportional to K_a , and q_{ba} is proportional to K_a and K_b . Since $K_b \ll K_a$, we can consider the q_{ba} term negligible in Eq. 3, i.e. adsorption of species a (serum) is independent of adsorption of species b (cytokine). The q_{ab} term in Eq. 4 gives rise to cytokine displacement by the higher relative affinity serum species. Our previously published single component model is a subset of the current model, where cytokine displacement is considered negligible ($q_{ab} \approx 0$).

2.4. Model Fitting to CLSM Data

Given the set of coupled equations describing mass transport of serum components and cytokine (Eq. 3 & 4, respectively), the unknown model parameters are:

$$\alpha_a = \frac{\rho q_a^{\max} R^2}{D_a c_a^{\text{in}} t_s} \quad \alpha_b = \frac{\rho q_b^{\max} R^2}{D_b c_b^{\text{in}} t_s} \quad K_a = \frac{c_a^{\text{in}}}{c_{50a}} \quad K_b = \frac{c_b^{\text{in}}}{c_{50b}}$$

We can separate known constants from the α terms: $\rho = 1.02 \text{ g/cm}^3$, $R = 266 \mu\text{m}$, $t_s = 21 \text{ hr}$. The remaining unknown parameters are:

$$\beta_a = \frac{q_a^{\max}}{D_a c_a^{\text{in}}} \quad \beta_b = \frac{q_b^{\max}}{D_b c_b^{\text{in}}} \quad K_a = \frac{c_a^{\text{in}}}{c_{50a}} \quad K_b = \frac{c_b^{\text{in}}}{c_{50b}}$$

The equation describing cytokine mass transport (Eq. 4) has three unknown parameters (K_a , K_b , β_b), but K_b and β_b only appear as a product, and therefore the two parameters cannot be fitted independently. A value of $\beta_b = 1 \times 10^{12} \text{ ml} \cdot \text{mg}_{\text{bead}}^{-1} \cdot \text{cm}^{-2} \cdot \text{s}$ was calculated as a reasonable estimate, using $c_b^{\text{in}} = 1 \mu\text{g/ml}$ (spiked IL-6 concentration), $q_b^{\max} = 1 \text{ mg/mg}_{\text{bead}}$ (order of magnitude approximation), and $D_b = 1 \times 10^{-9} \text{ cm}^2/\text{s}$ (calculated in previous CLSM work [12]); thereby permitting independent fitting of the remaining parameters.

The system of equations was solved for q_b (adsorbed cytokine) using 219 the finite element method with Comsol Multiphysics™, and a parameter optimization technique was developed as follows: numerical solutions from Comsol were imported into Matlab™, and the three unknown model parameters (β_a , K_a , K_b) were iteratively fit to IL-6 CLSM data by minimizing sum of squares error (SSE) between the numerical solutions and CLSM data

using a Nelder-Mead simplex algorithm: $\min_{p=\{\beta_a, K_a, K_b\}} \sum_{j=1}^{N_t} \sum_{i=1}^{N_r} [d_j(i) - q_b(i, t, p)]^2$, where N_t is the number of incubation time points, N_r is the number of radii data points, and $d_j(i)$ is the normalized CLSM intensity value at incubation time j and radius segment i . Parameters were fit to 2.5hr and 5hr, or 2.5hr, 5hr and 21hr CLSM data sets, and SSE was calculated as the cumulative error for all time points. CLSM data were normalized by the maximum intraparticle intensity values, and model simulations were normalized by the maximum intraparticle q_b values. CLSM intensities are directly proportional to adsorbed cytokine within the particle, therefore normalized CLSM and simulation data can be compared in this manner. Normalized CLSM intensity values [$d_j(i)$] and simulated adsorption profiles [$q_b(i, t, p)$] were multiplied by ten within the parameter optimization routine, such that the

normalized data ranged from zero to ten, rather than zero to one. This improved robustness of the error minimization algorithm, since the SSE calculation $[d_f(i) - q_b(i,t,p)]^2$ often resulted in extremely small values (approaching tolerances of the algorithm) when squaring the difference between values which were less than one..

2.5. Parametric Analysis

A parametric analysis was performed to determine (1) if multiple sets of parameter combinations existed that could fit the IL-6 CLSM data equally well, and (2) sensitivity of the model to parameter perturbations. Initial guesses for β_a , K_a , and K_b values were varied within a nominal range for each parameter, and best fit parameter values were 242 estimated for all combinations using the iterative error minimization algorithm (Table 2). Parameter sensitivity was examined by running model simulations using a subset range of parameter values (Table 3), and then plotting parameter values vs. SSE to quantify model sensitivity to parameter perturbations. All parametric analyses were run using solutions to the 2hr and 5.5hr time points.

3. RESULTS

3.1. Model Fits to Confocal Microscopy Data

Confocal laser scanning microscopy (CLSM) was used to quantify intraparticle adsorption profiles of fluorescently labeled IL-6 in horse serum. Baseline serum autofluorescence profiles for beads incubated only with horse serum for 2.5hr, 5hr, and 21hr are illustrated in Fig. 1(a). Various middle molecular weight serum solutes diffuse into the sorbent pores and adsorb to the polymer surface; certain adsorbed molecules autofluoresce in the wavelength range utilized during CLSM. Intraparticle intensity profiles for beads incubated with labeled IL-6 in horse serum are illustrated in Fig. 1(b). In contrast to adsorption behavior observed for baseline serum incubations, IL-6 profiles demonstrate peak intensities within the bead interior, suggesting competitive displacement phenomena [20]. Mean spatial intensities are shown from 4-5 beads imaged at each time point for both baseline and IL-6 incubations. Beads incubated in PBS were imaged to ascertain background signal from the bead itself, and intensities were negligible compared to those observed with baseline or IL-6 incubations (data not shown).

Baseline serum autofluorescence data (Fig. 1(a)) were subtracted from CLSM profiles for labeled IL-6 in serum (Fig. 1(b)), and resulting IL-6 profiles were used for model fitting. Model simulations were fit to normalized IL-6 CLSM data using an iterative parameter 265 optimization technique. Model fits for 2.5hr and 5hr time points are illustrated in Fig. 2(a) and 2(b), respectively. The corresponding best fit parameter values are shown in Table 1. Model predictions agree with the observed CLSM intraparticle cytokine profiles, indicating competitive adsorption between IL-6 and serum components. As IL-6 and serum solutes concurrently diffuse into the internal pore structure, high affinity serum components likely compete for binding sites with IL-6, leading to cytokine displacement within the particle. The model was also fit to IL-6 CLSM data for 2.5hr, 5hr, and 21hr incubation times (Fig. 3(a), 3(b), and 3(c), respectively). Addition of the 21hr time point did not have a substantial effect on best fit parameter estimates compared to the 2.5/5hr simulation, however, only the 2.5/5hr simulation was used for parametric analyses and low concentration simulations as this time scale is relevant to clinical use of the device. Discrepancies between model predictions and CLSM data at the bead surface ($r/R=1$) are likely due to imaging artifacts, as obtaining consistent CLSM intensity values in this region is technically challenging.

3.2. Parametric Analysis

A parametric analysis was performed to determine if multiple combinations of the three model parameters fit the CLSM data equally well. The parameter optimization routine was run using a range of initial parameter guesses (Table 2), and small differences in best fit parameter estimates were observed compared to those reported in Table 1. Variability of best fit parameter values was further examined to determine if these fluctuations were a result of multiple solutions to the model equations, or simply numerical tolerances of the error minimization algorithm. Parameter sensitivity was investigated by observing effects on model behavior 288 (quantified by SSE) due to perturbations in parameter inputs (Table 3). Fig. 4 illustrates model dependency on the three parameters (β_a , K_a , K_b). For each three dimensional graph, two parameters are plotted against SSE while the third parameter is held constant. A representative plot is shown for each parameter combination: K_a vs. K_b (Fig. 4(a)), K_a vs. β_a (Fig. 4(b)), K_b vs. β_a (Fig. 4(c)). For each case, eleven separate graphs were generated using values for the third variable which was not plotted, but model behavior was comparable between each of the eleven graphs for all cases. In Fig. 4(a-b), changes in K_a have minimal effects on SSE, indicating negligible dependence of the model on K_a . Fig. 4(c) illustrates model dependence on both K_b and β_a . A smaller slice of the K_b vs. β_a graph was investigated to determine if multiple local SSE minima existed that could result in best fit parameter variability using the SSE minimization algorithm. Fig. 5 illustrates a 100-fold smaller range of parameter values for K_b and β_a . Small fluctuations in SSE are observed, which may cause the error minimization algorithm to terminate in any of the local SSE minima wells. Therefore, small differences in best fit parameters based on initial parameter guess are most likely due to sensitivity of the SSE minimization algorithm, not multiple solutions to the model equations.

3.3. Physiologic Cytokine Concentration Simulation

The model was simulated for clinically relevant cytokine concentrations ($c_b^{\text{in}} = 1\text{ ng/ml}$) which were below the detection limit for CLSM analysis, using the best fit model parameter values estimated from IL-6 CLSM data at $1\mu\text{g/ml}$ IL-6 concentrations (Table 1; 2.5/5hr values). Fig. 6 illustrates model simulations for 2.5hr, 5hr and 21hr cytokine incubations at 1 ng/ml IL-6 concentration. Predicted adsorption profiles demonstrate single component diffusion/adsorption dynamics, in contrast to the displacement behavior observed for CLSM profiles at higher ($\sim 1\mu\text{g/ml}$) IL-6 concentrations. β_a , K_a , and K_b were varied to determine if small changes in parameter inputs affected predicted adsorption behavior at low cytokine concentration, however, the same qualitative adsorption patterns were observed for all parameter values tested (data not shown). These results indicate that coadsorption of serum solutes does significantly affect IL-6 adsorption dynamics under physiologic cytokine concentrations.

4. DISCUSSION

Modeling cytokine transport within the hemoadsorption beads is an important tool for optimizing clinical device parameters, and developing enhanced sorbent materials. In our original modeling work [13], we assumed that coadsorption of serum solutes within the beads would not affect cytokine capture dynamics. The goal of the present work was to directly test this hypothesis by quantifying competitive adsorption effects within single sorbent beads. We previously demonstrated that IL-6 intraparticle adsorption in the presence of PBS + 5% BSA was predicted by a single component diffusion/adsorption model [12]. In order to more closely mimic physiological conditions in this study, CLSM was performed with labeled IL-6 in horse serum, and resulting adsorption profiles indicated possible competitive adsorption behavior.

Sorbent CLSM studies are typically performed using simple protein solutions, where a molecule of interest is labeled with a fluorophore, and visualized within the particle using laser excitation at a specific wavelength [30]. In this study, we were interested in examining adsorption behavior within a biological fluid, and found that accumulation of adsorbed serum solutes within the sorbent particle caused autofluorescence profiles over a wide range of excitation wavelengths. We chose an excitation/emission wavelength (543/572nm) that minimized serum autofluorescence and maintained adequate signal from 334 the labeled IL-6. A simple signal processing technique was developed to subtract average serum autofluorescence signal from labeled IL-6 signal, and model simulations were fit to the post-processed IL-6 profiles. Although CLSM profiles of adsorbed serum components were quantified (Fig. 1(a)), we chose to fit model simulations only to adsorbed IL-6 profiles. Intraparticle profiles from serum autofluorescence varied greatly depending on wavelength used during CLSM, likely due to differing diffusional rates and autofluorescence emissions of serum solutes. These data were not suitable for model fitting, therefore we used the IL-6 adsorption profiles to fit model parameters corresponding to diffusion/adsorption of both IL-6 (K_b) and serum solutes (β_a , K_a).

The set of model equations contained four unknown parameters (β_a , K_a , β_b , K_b), however, the β_b term existed only as a product with K_b , and therefore these two parameters could not be fitted independently. A value of $\beta_b = 1 \times 10^{12} \text{ ml} \cdot \text{mg}_{\text{bead}}^{-1} \cdot \text{cm}^{-2} \cdot \text{s}$ was calculated as a

reasonable estimate $\left[\beta_b = \frac{q_b^{\max}}{D_b c_b^{\text{in}}} \right]$, using $c_b^{\text{in}} = 1 \mu\text{g/ml}$ (spiked IL-6 concentration), $q_b^{\max} = 1 \text{ mg/mg}_{\text{bead}}$ (order of magnitude approximation), and $D_b = 1 \times 10^{-9} \text{ cm}^2/\text{s}$ (calculated in previous CLSM work [12]). Reliable confidence interval estimates could not be obtained for the fitted parameters, therefore, we performed a parametric analysis to examine sensitivity of the model to parameter perturbations. Results indicate that a single combination of the three fitted parameters fits the IL-6 CLSM data. Small differences in best fit parameter estimates based on initial parameter guesses (Table 2) are likely due to numerical tolerances within the error minimization algorithm, given the local SSE minima observed over small ranges of parameter values (Fig. 5). A range of best fit parameter inputs (Table 2) was tested using the low concentration simulation, and no qualitative changes in predicted IL-6 adsorption profiles were observed from those illustrated in Fig. 6. Additionally, varying our β_b estimate did not have a meaningful effect on predicted low concentration IL-6 adsorption profiles (data not shown). Overall, our model fitting and parametric analysis results indicate that under clinically relevant cytokine concentrations ($c^{\text{in}} = 1 \text{ ng/ml}$), competitive adsorption effects within the hemoadsorption beads due to coadsorption of serum solutes do not significantly affect IL-6 adsorption dynamics.

Several important assumptions were necessary for development of the model framework and experimental techniques. We could not directly quantify the cytokine/fluorophore degree of labeling (DOL) due to low cytokine concentrations used, however, we previously demonstrated that changing the IL-6 DOL had negligible effects on intraparticle IL-6 transport [12], and assumed in this work that addition of the fluorophore tag did not significantly affect IL-6 adsorption dynamics. Results from other CLSM studies have supported this assumption [23], although altered solute adsorption due to fluorophore labeling has been reported as well [31]. Given the importance of solute size and hydrophobicity for retention within CytoSorb beads, labeled IL-6 could potentially possess altered physicochemical characteristics compared to native IL-6. We were unable to directly measure retention differences between the two species due to interference from the fluorophore tag within an IL-6 ELISA assay, however, our parametric analysis indicated that small changes in diffusivity (β_i) and affinity (K_i) do not substantially change the adsorption

behavior predicted for physiologic IL-6 conditions. As such, we would not expect small differences between native and labeled IL-6 to significantly affect results of this study.

The sorbent pore structure was specifically designed to exclude solutes larger than ~60kD (e.g. albumin, immunoglobulins), while allowing smaller solutes (e.g. cytokines, peptides) to diffuse and adsorb within the bead. In our model, we assumed a homogeneous pore size distribution and minimal effects from hindered diffusion or pore clogging; we used a spatially independent “effective” diffusion coefficient which accounted for tortuosity of the pore structure. The model could be enhanced through coupling a variable diffusion coefficient with adsorbed solute concentration, which would potentially offer a more realistic depiction of IL-6 and serum coadsorption. We would expect effects from hindered diffusion or pore clogging to become especially relevant at long incubation times as the sorbent becomes saturated, however, inclusion of the 21hr incubation time did not substantially change parameter estimates using the model (Table 1), indicating a temporally independent diffusion process within this timeframe. Given our interest in characterizing cytokine transport within a clinically relevant time scale (4-6hr), our model assumptions regarding solute diffusion seem appropriate.

Measurement of the Langmuir adsorption isotherm is useful for characterizing interactions between solutes and the sorbent surface, however, we found it impractical to measure the IL-6 isotherm due to the large amount of cytokine necessary to reach equilibrium within the bead. IL-6 must diffuse to the center of the particle to reach a time independent equilibrium state with the polymer surface, an extremely slow process, given that IL-6 diffuses only ~30% into the particle after 21hr (Fig. 3(c)). Even using low bulk IL-6 concentrations, the amount of cytokine necessary to generate the linear portion of the isotherm was prohibitive. For the purposes of this study, a lumped parameter approach was sufficient to characterize IL-6 adsorption dynamics, and to examine competitive adsorption behavior under physiologic conditions. We previously used similar lumped parameter modeling to characterize cytokine capture using the hemoabsorption beads [12-13], and found this approach to be a simple and useful methodology that could be applied to studies of other sorbent materials used in blood purification applications.

5.0 CONCLUSIONS

The work presented here describes a technique to quantify competitive protein adsorption effects within hemoabsorption beads in whole serum. Most studies of mass transport within sorbent particles are applicable to well characterized feed stocks, whereas the complexity of biological fluids requires new methodologies to examine mass transport phenomena. Increasing interest in sorbent-based blood purification technologies necessitates understanding of mass transport within these types of materials. Results from this study will assist in modeling effects of hemoabsorption therapy using our device on systems-wide inflammatory pathways in the critically ill, and help to elucidate new pathways towards development of enhanced sorbent materials.

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LIST OF SYMBOLS

$q_i(\mathbf{r},t)$	mass density of adsorbed species i per bead mass (mg/mg _{bead})
$c_i(\mathbf{r},t)$	mass concentration of species i within the liquid phase of the sorbent pores (mg/ml)
D_i	effective intraparticle diffusion coefficient of species i (cm ² /s)
R	average particle radius (μm)
q_i^{max}	maximum adsorbed mass of species i per bead 426 mass (mg/mg _{bead})
c_i^{in}	initial bulk concentration of species i (mg/ml)
t_s	time scale corresponding to experimental conditions used in the study (hr)
ρ	bead density (g/cm ³)
c_{i50}	concentration of species i necessary to saturate 50% of available sorbent binding sites (mg/ml)
K_a	relative affinity (c_a/c_{a50}) of serum component
K_b	relative affinity (c_b/c_{b50}) of IL-6

REFERENCES

- [1]. Venkataraman R, Subramanian S, Kellum JA. Critical Care. 2003; 7:139–145. [PubMed: 12720560]
- [2]. Kellum JA, Song M, Venkataraman R. Crit Care Med. 2004; 32:801–805. [PubMed: 15090965]
- [3]. Peng Z, Carter M, Kellum JA. Crit Care Med. 2008; 36:1573–1577. [PubMed: 18434884]
- [4]. Zhou X-P, Li W, Shi Q-H, Sun Y. Journal of Chromatography A. 2006; 1103:110–117. [PubMed: 16313916]
- [5]. Yang K, Shi Q-H, Sun Y. Journal of Chromatography A. 2006; 1136:19–28. [PubMed: 17034803]
- [6]. Noriega JA, Tejeda A, Magana I, Ortega J, Guzman R. Biotechnol. Prog. 1997; 13:296–300.
- [7]. Horstmann BJ, Chase HA. Bioseparation. 1998; 7:145–157. [PubMed: 10036752]
- [8]. Bak H, Thomas ORT, Abildskov J. Journal of Chromatography B. 2007; 848:131–141.
- [9]. House AH, Ronco C. Blood Purification. 2008; 26:30–35. [PubMed: 18182792]
- [10]. Li L, Pan J, Yu Y. Biomed. Mater. 2007; 2:R12–R16. [PubMed: 18458435]
- [11]. Winchester J, Kellum JA, Ronco C, Brady JA, Quartararo PJ, Salsberg JA, Levin NW. Blood Purification. 2003; 21:79–84. [PubMed: 12566666]
- [12]. Kimmel JD, Gibson GA, Watkins SC, Kellum JA, Federspiel WJ. J Biomed Mater Res B Appl Biomater. 2010; 92:390–396. [PubMed: 19904819]
- [13]. DiLeo MV, Kellum JA, Federspiel WJ. Annals of Biomedical Engineering. 2009; 37:222–229. [PubMed: 18949559]
- [14]. DiLeo MV, Fisher JD, Federspiel WJ. Annals of Biomedical Engineering. 2009; 37:2310–2316. [PubMed: 19680812]
- [15]. Gallant SR. Journal of Chromatography A. 2004; 1028:189–195. [PubMed: 14989472]
- [16]. Linden T, Ljunglof A, Kula MR, Thommes J. Biotechnology and Bioengineering. 1999; 65:622–630. [PubMed: 10550768]
- [17]. Finette GM, Baharin BS, Mao Q, Hearn MT. Biotechnol. Prog. 1997; 13:265–275. [PubMed: 9190077]
- [18]. Skidmore GL, Chase HA. Journal of Chromatography A. 1990; 505:329–347.
- [19]. Lewus RK, Carta G. AIChE Journal. 1999; 45:512–522.
- [20]. Martin C, Iberer G, Ubiera AR, Carta G. Journal of Chromatography A. 2005; 1079:105–115. [PubMed: 16038296]

- [21]. Brooks CA, Cramer SM. *AIChE Journal*. 1992; 38:1969–1978.
- [22]. Ljunglof A, Hjorth R. *Journal of Chromatography A*. 1996; 743:75–83.
- [23]. Linden T, Ljunglof A, Hagel L, Kula MR, Thommes J. *Separation Science and Technology*. 2002; 37:1–32.
- [24]. Hubbuch J, Linden T, Knieps E, Thommes J, Kula MR. *Biotechnology and Bioengineering*. 2002; 80:359–368. [PubMed: 12325144]
- [25]. Hubbuch J, Linden T, Knieps E, Ljunglof A, Thommes J, Kula MR. *Journal of Chromatography A*. 2003; 1021:93–104. [PubMed: 14735978]
- [26]. Ljunglof A, Bergvall P, Bhikhabhai R, Hjorth R. *Journal of Chromatography A*. 1999; 844:129–135. [PubMed: 10399329]
- [27]. Ljunglof A, Thommes J. *Journal of Chromatography A*. 1998; 813:387–395. [PubMed: 9700931]
- [28]. Harinarayan C, Mueller J, Ljunglof A, Fahrner R, Van Alstine J, van Reis R. *Biotechnology and Bioengineering*. 2006; 95:775–787. [PubMed: 16897740]
- [29]. Song M, Winchester J, Albright R, Capponi V, Choquette M, Kellum JA. *Blood Purification*. 2004; 22:428–434. [PubMed: 15316198]
- [30]. Hubbuch J, Kula MR. *Bioprocess Biosyst Eng*. 2008; 31:241–259. [PubMed: 18196281]
- [31]. Teske C, Von Lieres E, Schroder M, Ladiwala A, Cramer S, Hubbuch J. *Biotechnology and Bioengineering*. 2006; 95:58–66. [PubMed: 16752371]

Fig. 1.

(a) CLSM intraparticle intensity profiles for beads incubated with horse serum for 2.5hr, 5hr, and 21hr. (b) CLSM intraparticle intensity profiles for beads incubated with fluorescently labeled IL-6 in horse serum for 2.5hr, 5hr, and 21hr. Average baseline serum autofluorescence values were subtracted from IL-6 intensity data to separate background serum signal from IL-6 adsorption profiles for model fitting. Error bars indicate standard deviation from 4-5 beads imaged at each time point.

Fig. 2.

The two component model was fit to IL-6 intraparticle intensity curves using a parameter optimization technique. Model fits (solid lines) are shown for (a) 2.5hr and (b) 5hr CLSM incubation time points. Normalized data was scaled by a factor of ten to 493 ensure that sum of squares error (SSE) was greater than numerical tolerances of the error minimization algorithm.

Fig. 3. Model fits (solid lines) to IL-6 CLSM data for (a) 2.5hr, (b) 5hr and (c) 21hr incubation time points. Normalized data was scaled by a factor of ten to ensure that sum of squares error (SSE) was greater than numerical tolerances of the error minimization algorithm.

Fig. 4. Sensitivity of the model to parameter perturbations. Three dimensional plots are shown for (a) K_a vs. K_b , (b) K_a vs. β_a , and (c) K_b vs. β_a , plotted against SSE.

Fig. 5. Model sensitivity to small changes in K_b and β_a . Variations in SSE error are observed, with multiple local SSE minima existing throughout the parameter space.

Fig. 6. Model simulations for low cytokine concentration incubations ($c^{\text{in}} = 1\text{ ng/ml}$). In contrast to the behavior observed at high IL-6 concentrations, low concentration simulations predict classical single component adsorption/diffusion behavior with negligible competitive adsorption effects.

Table 1

Best fit model parameters based on sum of squares error (SSE) minimization between model simulations and IL-6 CLSM data.

	K_a	K_b	β_a (ml·mg ⁻¹ ·cm ⁻² ·s)	SS Error
2.5hr, 5hr	4.43	1.43e-5	6.82e10	115.4
2.5hr, 5hr, 21hr	5.10	9.43e-6	6.89e10	341.8

Table 2

Initial guess parameter values used within the parameter optimization routine, and resulting range of best fit parameter estimates.

	Initial parameter guesses		Best fit parameter estimates	
	low	high	low	high
K_a	1	10	2.4	3.8
K_b	5e-6	1e-3	1.3e-5	1.52e-5
βa (ml·mg ⁻¹ ·cm ⁻² ·s)	3e10	50e10	7.27e10	9.56e10

Table 3

Range of parameter inputs used to test parameter sensitivity of the model. Eleven equidistant parameter values were used within each specified parameter range.

	Parameter ranges	
	low	high
K_a	3.5	4.5
K_b	0.5e-5	10e-5
β_a (ml·mg ⁻¹ ·cm ⁻² ·s)	3e10	50e10