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Chemical-PDMS Binding Kinetics and Implications for Bioavailability in Microfluidic Devices

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Abstract

Microfluidic organ-on-chip devices constructed from polydimethylsiloxane (PDMS) have proven useful in studying both beneficial and adverse effects of drugs, supplements, and potential toxicants. Despite multiple advantages, one clear drawback of PDMS-based devices is binding of hydrophobic chemicals to their exposed surfaces. Chemical binding to PDMS can change the timing and extent of chemical delivery to cells in such devices, potentially altering dose-response curves. Recent efforts have quantified PDMS binding for selected chemicals. Here, we test a wider set of nineteen chemicals using UV-Vis or infrared spectroscopy to characterize loss of chemical from solution in two setups with different PDMS-surface-to-solution-volume ratios. We find discernible PDMS binding for eight chemicals and show that PDMS binding is strongest for chemicals with a high octanol-water partition coefficient ($\text{Log } P > 1.85$) and low H-bond donor number. Further, by measuring depletion and return of chemical from solution over tens to hundreds of hours and fitting these results to a first order model of binding kinetics, we characterize partitioning into PDMS in terms of binding capacities per unit surface area and both forward and reverse rate constants. These fitted parameters were used to model the impact of PDMS binding on chemical transport and bioavailability under realistic flow conditions and device geometry. The models predict that PDMS binding could alter in-device cellular exposures for both continuous and bolus dosing schemes by up to an order of magnitude compared to nominal input doses.

Graphical Abstract

We measure chemical-PDMS binding for 19 chemicals, correlate binding with molecular properties, and use measured kinetics to model in-device bioavailability.

Conflicts of interest

There are no conflicts of interest to declare.

Introduction

Microfluidic organ-on-chip devices have proven useful in studying both beneficial and adverse effects of drugs, supplements, and potential toxicants through improved response times and reduced costs in bioactivity screens.¹ Such devices have also been used to investigate chemical effects in models for a range of biological systems and processes: e.g., mammary glands;² lungs;³ hepatotoxicity;⁴ renal differentiation;⁵ and multi-organ coupling.⁶ The primary polymer used to fabricate microfluidic devices has been polydimethylsiloxane (PDMS). The advantages of PDMS range from its optical transparency to its gas permeability to its ease of fabrication.⁷ In addition, compared to rigid glass or plastic substrates, PDMS-based devices provide cultured cells with a more porous and less stiff mechanical environment – still artificial, but closer in mechanical properties to soft tissues.^{8,9}

Despite these advantages, one clear drawback of PDMS is its hydrophobicity. This disadvantage is particularly worrisome in chemical screening applications because hydrophobic compounds can bind to or become sequestered within PDMS. Such binding causes a discrepancy between the nominal inlet concentration and actual cellular exposures, affecting dose-response curves.¹⁰ The aims of this study are to characterize those chemical properties that are predictive of PDMS binding, to present a simple protocol for experimentally measuring the on- and off-rate PDMS-binding kinetics, and to show how the measured kinetic parameters can be used to model chemical transport in PDMS-based devices to predict and/or design actual cellular exposures.

The binding and sequestration of hydrophobic compounds by PDMS was first investigated qualitatively using fluorescent molecules.^{40,41} Subsequent quantitative studies suggested relationships between a compound's degree of PDMS binding and its octanol/water partition coefficient (LogP) or its topological polar surface area (TPSA). One study suggested a LogP threshold – strong binding for highly hydrophobic compounds with LogP > 2.62.⁴² The follow-up, which only tested compounds above the LogP threshold, suggested a linear correlation of stronger binding with smaller TPSA.⁴³ These two studies were limited to evaluation of just 5 and 4 compounds, respectively. To further investigate the link between molecular properties and chemical partitioning into PDMS, we have chosen a larger, more diverse sample of 19 test compounds. These chemicals have a range of uses – from pesticides to pharmaceuticals to the manufacture of consumer products – and were selected due to their use in current organ-on-chip toxicology studies. As detailed in Table 1, many of

the test compounds have been linked to endocrine disruption and developmental or reproductive toxicity; others serve as negative controls with no known toxicity in mammals. To interpret the results of toxicology studies using these compounds in PDMS-based microfluidic devices, it is crucial that we can accurately predict their in-device bioavailability.

Such predictions will rely on computational models. Here we provide a simple method for measuring the needed model parameters for reversible and irreversible PDMS-binding kinetics. These include the forward and backward rate constants, as well as chemical-specific carrying capacities per unit of PDMS surface area. Previous approaches to this problem explicitly modelled diffusional transport of chemicals within PDMS;⁴⁴ however, we find that the combined effects of partitioning at the solution-PDMS interface and diffusion into the PDMS bulk are well described by rate constants and carry capacities over tens to hundreds of hours. For all but one tested compound, we used time-resolved UV-Vis absorption spectroscopy to monitor depletion (and later return) of chemical from (to) a solution in contact with either a PDMS disk or the walls of a PDMS microfluidic channel. The exception was perfluorooctanoic acid (PFOA), which had insufficient light absorption within an accessible UV-Vis spectral window. As an alternative, we measured PFOA's infrared absorption in attenuated total reflectance (ATR) mode to directly measure its accumulation on PDMS surfaces. Once the binding parameters are measured, we then present a model that combines computational fluid dynamics (CFD) with PDMS-binding kinetics to predict chemical bioavailability in a simple microfluidic device. These predictions include temporally- and spatially-varying chemical concentrations in the perfusion media, as well as the effective surface density of bound chemical throughout the device. We use this model to highlight what typical PDMS-binding parameters imply for bioavailability. Our approach complements prior work that focused on microfluidic design considerations for minimizing the impact of sequestration in PDMS.⁴⁴ These design considerations depended on the properties of the chemicals to be tested – *e.g.*, partition and diffusion coefficients – and are thus not as useful when designing a single microfluidic system to test a wide range of drugs or potential toxicants. Explicitly modeling bioavailability for each chemical is thus a key step towards pharmacokinetics for organ-on-chip or microphysiological systems.

Experimental Design

PDMS Preparation

PDMS Sylgard 184 (Dow Corning, Auburn, MI) was mixed with a 1:10 weight ratio of curing agent to elastomer. For disk-soak experiments, PDMS was cast in a 5-mm thick layer, cured for 24 hours, and cut into cylindrical disks (6-mm in diameter). For channel-soak experiments, PDMS was cast in a 3-mm thick layer over a channel mould, which was fabricated using standard photolithography on a Si wafer with SU8-2050 photoresist. After curing the PDMS for 24 hours, inlets were formed by punching 1.5-mm diameter cylindrical reservoirs at both ends of the channel. To reversibly seal channels for long-duration chemical exposures, channel-containing PDMS layers were sandwiched between two other 3-mm

thick PDMS layers and subjected to continuous pressure from a weighted plate. Channel dimensions are given in Fig. 1B.

Chemical Preparation

All chemicals were purchased in powder form (except liquid molinate) from Sigma Aldrich (Saint Louis, MO). Chemicals to be tested were dissolved in either molecular biology grade water or a 1X phosphate buffered saline (PBS) solution with added dimethyl sulfoxide (DMSO) to increase solubility of hydrophobic compounds ($\text{LogP} > 1$). For most chemicals, the final DMSO fraction was 0.1%, but 10% was used for docetaxel. Chemicals were diluted in their respective solvent to starting concentrations that yielded a peak UV-Vis absorbance of one or as close as solubility allowed. Molecular properties cited here were from the EPA Chemistry Database,³⁷ Canadian Institute of Health Toxin Database,⁴⁵ University of Hertfordshire Pesticide Properties Database,⁴⁶ and select publications for genistein.⁴⁷ For most chemicals, cited LogP values were from database-reported experimental measurements; for three chemicals (PFOA, formetanate, and SDG) experimental values were not available and we instead cite database-reported values for calculated LogP (cLogP).

Assessing PDMS Binding via UV-Vis Measurements

For disk-soak experiments, we conducted both on- and off-rate experiments. On-rate experiments were designed to measure the rate at which each chemical partitioned out of solution and onto or into a PDMS disk. In brief, each sample solution was placed in a 4-ml quartz cuvette and a PDMS disk was carefully placed on the surface. Due to the relative densities of water and PDMS, such disks float with a reproducible volume above and below the surface. Cuvettes with disks were placed on an orbital shaker to keep solutions well mixed. Periodically, cuvettes were moved to a UV-Vis spectrometer to have spectra measured with disks still floating above the spectrometer light beam (Fig. 1A). Chemical binding to the floating PDMS disk was tracked via depletion of chemical from solution. At the end of an on-rate experiment, if there was evidence of chemical binding to the PDMS disk, then the disk was removed from its sample-solution cuvette, dried with gaseous nitrogen, and carefully floated on the surface of fresh solvent in a new cuvette (Fig. 1A). Shaking and periodic UV-Vis measurements were then performed as above to conduct an off-rate experiment that tracked the rate at which surface-bound chemical partitioned back off the PDMS disk and into solution.

UV-Vis absorbance spectra for on- and off-rate experiments were measured against matched cuvettes with appropriate solvent using a Cary 5000 dual-beam UV-Vis spectrometer (Agilent, Santa Clara, CA; scan rate = 24 nm/min; resolution = 1 nm). To control for chemical stability, spectra were also measured periodically for positive control cuvettes containing sample solutions without PDMS disks. To correct for instrumental baseline drift, spectra were concomitantly measured for negative control cuvettes containing appropriate solvent only.

For channel-soak experiments, each channel was filled with a chemical solution and pressure-sealed against other PDMS layers. After a pre-determined soak time, the channel

was opened, the chemical solution was pipetted out, and its UV-Vis spectrum was measured using a Nanodrop 2000C Spectrophotometer (Thermo Fisher, Waltham, MA). Time-resolved measurements were thus obtained by sealing individual channels for different periods of time. Each time-point measurement was repeated in triplicate.

To convert UV-Vis absorbance to chemical concentration, a clearly discernible peak of interest was selected from spectra of each chemical at several dilutions and used to construct a linear calibration curve (measured independently for each spectrometer and in triplicate for each chemical).

Assessing PDMS Binding via FTIR Measurements

The UV-Vis absorption band for one tested chemical, PFOA, was too near the edge of UV detection for reliable measurement. As an alternative, we took IR spectra to measure PFOA bound to PDMS disks using a Nicolet IS5 FTIR spectrometer (Thermo Fisher, Waltham, MA) with a single-reflection diamond ATR attachment. Measurements were averaged 100 times with a resolution of 4 cm^{-1} and with the evanescent wave covering a 1.5-mm diameter area. For these measurements, PDMS disks were floated as detailed above in a solution of PFOA for 48 hours, removed from solution, dried with nitrogen, and placed directly onto the diamond ATR. Both PFOA-soaked disks and control solvent-soaked disks were measured in triplicate to confirm homogeneity of surface binding. Since FTIR spectra were measured at a single time point, they were only used to estimate the amount of PFOA bound and not its binding kinetics. To convert from IR absorbance to concentration, we used the strong PFOA vibrational mode at 1209 cm^{-1} , which corresponds to a $(\text{CF}_2)+(\text{CF}_3)$ asymmetric stretch,⁴⁸ and measured calibration spectra of diluted PFOA solutions in pure DMSO. The contribution of PDMS to FTIR spectra of soaked disks was minimized by weighted subtraction of a spectrum of a control solvent-soaked disk and a constant baseline offset, with weights determined by least squares minimization of the resultant spectrum in a region with no PFOA vibrational bands ($990\text{--}1040\text{ cm}^{-1}$).

Computational Model

Modelling of chemical transport in a microfluidic device, including binding and desorption from PDMS surfaces, was conducted using COMSOL Multiphysics (Burlington, MA). The modelled geometry was a single longitudinal plane through a simple rectangular microchannel (length = 8 mm, width = 1.5 mm, and height = 0.1 mm). Since channel width was much greater than height, variations in velocity and concentration along the channel width were neglected and a well-developed parabolic flow velocity was imposed vertically. Symmetry allowed for a reduction in computation time by explicitly modelling only the bottom half of the channel. Conditions were assumed to be isothermal, with convective flux boundary conditions specified at both device inlet and outlet. The model scheme was validated by simulating disk-soak experiments under well-mixed conditions to reproduce the experimental binding and desorption kinetics.

Results

The primary method used here to measure chemical binding to PDMS was quantifying the loss of chemical from a solution in contact with a PDMS disk or channel surface using UV-Vis absorbance (Fig. 1). Control experiments on matched solutions without PDMS disks confirmed that all but one tested chemical had no significant PDMS-independent loss from solution. That exception was molinate, likely due to its high volatility.⁴⁶ Its loss from control samples was measured and its binding to PDMS was assessed as the excess depletion observed in disk-soak experiments.

In disk-soak experiments, we observed no PDMS binding for any tested chemical with LogP < 2.5. On the other hand, four of the seven most hydrophobic chemicals tested in these experiments were lost from solution following exponential decays over tens of hours – see Fig. 2 for molinate, ethofumesate, propiconazole, and to a lesser degree, bisphenol A. This behaviour was not universal: other hydrophobic chemicals with LogP > 2.5, such as diethylstilbestrol, genistein and rhodamine 6G, showed no evidence of depletion from solution and thus no binding to PDMS. The most hydrophobic compound tested, PFOA, had no appropriate UV-Vis absorption, which precluded measuring its binding kinetics, but we were able to measure the degree to which it bound PDMS at a single time point using ATR-FTIR spectroscopy. We found that 24% of the PFOA originally in solution had bound to the surface of a PDMS disk after soaking for 48 hours.

When pre-soaked PDMS disks were transferred to fresh solvent, we found that two tested chemicals desorbed from PDMS and returned to solution: molinate and ethofumesate. As shown in Fig. 3, their desorption followed a roughly exponential approach to a new equilibrium between bound and free chemical. Molinate never reached a steady level, but instead appears to decrease after 40 hours because the correction for its PDMS-independent loss could not be implemented for off-rate experiments. Nonetheless, about 1/4 and 1/3 of the molinate and ethofumesate bound to a PDMS disk respectively returned to solution within 48 hours. The other two chemicals for which we could measure PDMS-binding kinetics, namely propiconazole and bisphenol A, bound irreversibly with no evidence of desorption in fresh solvent.

To empirically quantify the PDMS-binding kinetics of each chemical, we fit the disk soak results to exponential approaches to equilibrium:

$$A = A_0 + \Delta A_1 \left(1 - e^{-t/\tau_1}\right) \quad (1)$$

$$A = \Delta A_2 \left(1 - e^{-t/\tau_2}\right) \quad (2)$$

Eqn. 1 fits experiments in which an initial amount of chemical A_0 is depleted from solution with time constant τ_1 to approach a final value of $A_0 + \Delta A_1$ (in which $\Delta A_1 < 0$). Eqn. 2

similarly fits experiments in which an amount of chemical $A_2 > 0$ returns to solution as it desorbs from a pre-soaked disk with time constant τ_2 . For each chemical that bound PDMS, Table 2 lists the time constants (τ_1, τ_2), the fraction bound at equilibrium, $f_{B,eq} = -A_1/A_0$, and the fraction eventually returned to solution, A_2/A_0 . The “fraction bound” is also listed for chemicals that did not exhibit significant loss from solution for which it is based solely on the percent change in concentration between the start and end of experiments. Some of the non-binding chemicals have experimentally estimated values of $f_{B,eq}$ that are negative, but these are all within a few standard deviations of zero. Note that these are empirical descriptors specific to the stated experimental conditions.

Notably, rhodamine B did not show significant binding to PDMS in disk-soak experiments despite visibly dyeing the disk surface. Since rhodamine B has a high extinction coefficient, this visible dyeing could result from a very small amount bound. To quantify binding for chemicals like rhodamine B that partitioned into PDMS to a lesser extent, we thus conducted additional experiments in which solutions were sealed inside a microfluidic channel (dimensions as in Fig. 1). These channel-soak experiments had a much larger surface-to-volume ratio (116 cm^{-1} versus 0.3 cm^{-1}), which allowed detection of less extensive binding. Results from both types of experiments are compared in Table 2. Due to the shorter effective pathlength of the Nanodrop spectrophotometer, several chemicals had too little absorbance even at their solubility limit to have their PDMS binding measured using channel-soak experiments (diethylstilbestrol, propiconazole, molinate, ethofumesate, docetaxel).

Between the two sets of experiments, nine chemicals in our test set measurably bound to PDMS: PFOA, rhodamine 6G, propiconazole, bisphenol A, molinate, ethofumesate, rhodamine B, imazaquin and hexazinone. All had high LogP (> 1.8) and low TPSA ($< 91.6 \text{ \AA}^2$). These results are consistent with data from two previous studies by Wang *et al.* and Van Meer *et al.* that linked PDMS absorption to measures of high hydrophobicity.^{42,43} On the other hand, our larger test set identified several chemicals with similarly high LogP and/or low TPSA that did not measurably bind to PDMS, e.g., diethylstilbestrol and genistein (LogP of 5.07 and 3.04 respectively; see Table 2). We thus investigated whether any additional molecular property would distinguish the hydrophobic non-binders. The only combination we found that discriminated binders from non-binders was LogP and the number of H-bond donors. This discrimination is shown in Fig. 4A-B, which separately compare results for disk-soak experiments and channel-soak experiments. Previous studies had surface-to-volume ratios closer to our channel-soak experiments and are thus reported alongside those results in Fig. 4B. Whenever there was a discrepancy in reported LogP values, we plotted data points at both values and connected them with a horizontal line. There is clearly a threshold LogP (in the range of 1.27–1.85), below which chemicals do not bind PDMS. Above this threshold, chemicals may bind PDMS, but the strength of this binding decreases for molecules having more H-bond donors. An exception to this trend was rhodamine 6G as tested by Wang *et al.*¹¹ We tested rhodamine 6G in both our experimental setups and found very little binding to PDMS. This discrepancy will be revisited in Discussion.

Predicted Impact of Chemical-PDMS Binding.

As noted above, the empirical descriptors of PDMS binding are useful, but specific to limited experimental conditions. To find parameters more useful for modelling chemical-PDMS interactions over a wider range of concentrations and PDMS surface areas, we fit the data to a microscopic model of 1st order binding kinetics. This model considers a reaction between chemical A and PDMS-surface site S , i.e., $A + S \rightleftharpoons A_{bound}$. Allowing for reversible interactions, the reaction kinetics follow

$$\frac{d[A]}{dt} = -k_F[A][S] + k_R[A_{bound}] \quad (3a)$$

where brackets denote concentrations and k_F , k_R are the forward and backward rate constants. Dropping the brackets, making the time-dependent terms explicit, and casting $[S]$ and $[A_{bound}]$ in terms of $A(t)$ yields:

$$\frac{dA(t)}{dt} = -k_F A(t) \left(\frac{S_0}{\alpha} - (A_{tot} - A(t)) \right) + k_R (A_{tot} - A(t)) \quad (3b)$$

where A_{tot} is the total amount of chemical divided by the solution volume, S_0 is the initial surface density of binding sites, and α is the ratio of solution volume to PDMS surface area. For a given chemical, binding and desorption experiments were fit simultaneously with shared parameters. Binding experiments were fit to analytic solutions to Eqn. 3b using boundary condition $A(0) = A_{tot}$ = the stated starting concentration. Desorption experiments were fit to solutions with $A(0) = 0$ and A_{tot} being a concentration equivalent to desorbing all chemical bound to the disk's surface in the previous on-rate binding experiment. For chemicals that bound irreversibly, the desorption experiment was simply taken to yield $k_R = 0$. Microscopic model fits are shown alongside the empirical fits of binding/desorption kinetics in Figs. 2 and 3. Parameters from the microscopic model fits are compiled in Table 3.

These microscopic model fit parameters were then used in a computational fluid dynamics (CFD) model combining mass transport and surface reactions to predict the sequestration of chemicals in a PDMS-based microfluidic device (geometric details under Experimental Design). This model is very similar to those used in biosensor applications.^{49–53} Chemical transport in the bulk fluid is described by a convection-diffusion equation:

$$\frac{\partial c}{\partial t} = D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) - \frac{\partial c}{\partial x} \cdot \vec{u} \quad (4)$$

where c is the time-dependent chemical concentration, D is diffusivity of a chemical species in bulk fluid, and u is the position- and time-dependent fluid velocity. Chemical transport and reaction on the PDMS surface are governed by a reaction-diffusion equation:

$$\frac{\partial c_s}{\partial t} = D_s \left(\frac{\partial^2 c_s}{\partial x^2} + \frac{\partial^2 c_s}{\partial y^2} \right) + k_F c (S_0 - c_s) - k_R c_s \quad (5)$$

where c_s is the bound species surface density, D_s is its surface diffusivity, S_0 is the binding capacity per unit of PDMS surface area, and k_F , k_R are the forward and backward rate constants for surface binding respectively. The surface reaction expression in Eqn. 5 includes the bulk concentration, c , at the reacting surface. This coupling with mass balance in the bulk is obtained at the flux boundary according to

$$D \left(\frac{\partial c}{\partial y} \right) = k_F c (S_0 - c_s) - k_R c_s \quad (6)$$

Although this model does not explicitly consider diffusion into bulk PDMS, such diffusion must occur, especially for chemicals for which PDMS has a larger binding capacity. For example, the fitted binding capacity for molinate is 2500 molecules per nm^2 . If molinate molecules were truly packed on a PDMS surface at this density, each molecule would occupy an area of just $4 \times 10^{-4} \text{ nm}^2$, which is much too tightly packed to be reasonable. Instead, molinate and other chemicals with $S_0 > O(1 \text{ nm}^{-2})$ must penetrate into PDMS and S_0 should be considered an effective density per unit of geometric surface area.

To explore the potential range of binding and chemical sequestration in a microfluidic device, we ran this model for three tested chemicals: ethofumesate, which binds reversibly; propiconazole, which binds irreversibly; and rhodamine B, which is minimally adsorbed by PDMS. Since these chemicals were of similar size, their diffusivities were taken to be the same: $10^{-9} \text{ m}^2/\text{s}$ in aqueous solution (D) and $10^{-11} \text{ m}^2/\text{s}$ along the PDMS surface (D_s). Each model considered parabolic flow with a maximum velocity, $u_{max} = 100 \text{ } \mu\text{m}/\text{s}$. We model a hypothetical device in which a cell culture chamber begins at the end of an 8-mm long channel; cellular exposure is thus taken as the chemical concentration just above the PDMS surface at the end of this channel. We investigated effects under both continuous injection of chemicals (starting from $t = 2$ hours) and bolus injections (from $t = 2$ to 6 hours) over a wide range of inlet concentrations from 10^{-2} to 10^{-7} M . Inlet concentrations for ethofumesate and propiconazole were limited to 10^{-4} M due to their low aqueous solubility. Predicted cellular exposures are shown for all three chemicals for continuous and bolus injections in Fig. 5A-C and 6A-C, respectively. These figures also include the corresponding degree to which PDMS binding sites are saturated (Fig. 5D-F, 6D-F).

Under continuous dosing, the differential impacts of reversible and irreversible PDMS binding can be seen by comparing Fig. 5A,D and 5B,E. For a reversible binder like ethofumesate, the predicted cellular exposure gradually increases with time and asymptotically approaches the inlet concentration (Fig. 5A). This occurs for all inlet concentrations once the on- and off-rates for PDMS binding approach equilibrium. For the highest inlet concentration simulated (10^{-4} M), this equilibrium occurs at nearly 70% surface saturation (Fig. 5D). On the other hand, for an irreversible binder like propiconazole,

the predicted cellular exposures only approach the nominal inlet concentrations once the surface becomes fully saturated (Fig. 5B,E). Even at the highest dose simulated (10^{-4} M), reaching saturation can take several hundred hours. For doses that do not yield surface saturation within the simulated time window (200 h), the predicted cellular exposure remains an order of magnitude less than the nominal inlet concentration.

Additional impacts arise under bolus dosing. For a reversible binder like ethofumesate, exposure was at lower levels (less than 30% of inlet exposure) for all of the 4 hour bolus period (Fig. 6A,D). Even more interestingly, once a bolus dose ended, cellular exposure continued. This extended exposure was due to gradual chemical desorption from the surface. It could initially be as large as 5% of the bolus exposure and gradually diminished to less than 1% after 48 hours. Such extended exposures were absent for an irreversible binder like propiconazole, but it too had effects that were highly dependent on the nominal inlet concentration. Only at the highest simulated dose (10^{-4} M) was propiconazole able to saturate the device's PDMS surfaces during the bolus period and thus yield cellular exposures approaching the nominal inlet concentration. For all other simulated doses, the exposures were an order of magnitude less than the nominal dose.

For weaker binding chemicals like rhodamine B, cellular exposures closely match inlet concentrations. This is true for all simulated doses under both continuous (Fig. 5C,F) and bolus exposures (Fig. 6C,F). At low inlet concentrations (10^{-7} to 10^{-5} M), the on-rate for binding is so low that there is little impact on cellular exposures under the modelled flow conditions. At higher inlet concentrations (10^{-2} to 10^{-4} M), binding is more rapid and the system quickly reaches surface saturation (Fig. 5F), but the low binding capacity of the surface again results in little change in chemical concentrations throughout the perfusate.

Discussion

Here we have investigated binding to PDMS surfaces for 19 chemicals of interest in environmental toxicology. This set of test chemicals covers a wider range of molecular properties than previous studies and allows us to further delineate those characteristics most closely associated with binding to PDMS. In addition, for those chemicals that did bind, we have more fully characterized the on- and off-rate kinetics to facilitate predictive modelling of chemical sequestration and actual cellular exposures in PDMS-based microfluidic devices.

Importantly, we used two experimental setups, disk soaks and channel soaks, to fully characterize both weak and strong PDMS affinities. Only one compound in our test set, i.e., bisphenol A, was amenable to kinetic characterization in both setups. Given the different surface-to-volume ratios and starting concentrations, the two experiments for bisphenol A yielded quite different empirical parameters (% bound and time constants); however, all of the bisphenol A data could be fit well simultaneously with a single set of microscopic kinetic parameters (k_F , k_R and S_0) (Fig. S1). This consistency is an important validation of the approach taken here.

In terms of the molecular properties that influence PDMS binding, we also find a key role for measures of chemical hydrophobicity. Wang *et al.* tested five compounds and noted an apparent LogP threshold separating chemicals that bound PDMS strongly (> 2.62) from those that did so weakly or not at all (< 2.47).⁴² Van Meer *et al.* tested four other chemicals – all with LogP above the apparent threshold – and instead noted a linear correlation between the percent remaining unbound to PDMS and the compounds' TPSA, another measure of hydrophobicity.⁴³ Once we add our data, these measures are no longer fully predictive of PDMS binding over the combined data set of 26 chemicals. We find that insufficient hydrophobicity is still a useful predictor of chemicals that do not partition into PDMS. Both LogP and TPSA can be used to establish such a threshold at less than 1.85 for LogP or greater than 91.6 Å² for TPSA. Note that these results were obtained using unmodified PDMS; plasma treatments used to reduce PDMS surface hydrophobicity could alter the LogP and TPSA binding thresholds.

Despite agreement on thresholds, we find that the degree of PDMS binding for chemicals with LogP above (or TPSA below) threshold is no longer linearly related to TPSA. Neither is it related to molecular weight (range from 187 to 808 g/mol) or polarizability (range from 17 to 980 Å³). Among those and 15 other molecular properties catalogued by ChemSpider (<http://www.chemspider.com>), the best predictor of PDMS binding was LogP above the noted threshold and the number of H-bond donor groups (T-test P-value = 0.0037). Highly hydrophobic compounds with no H-bond donor groups were strongly sequestered by PDMS, those with one tended to be sequestered more modestly, and those with two or more were affected weakly if at all. One chemical right at the logP threshold (hexazinone) with zero H-bond donors did not partition appreciably into PDMS in disk-soak experiments, but did so in channel soaks.

As noted above in Results, the large PDMS-binding capacities (S_0) for some chemicals show that substantial sequestration requires both surface partitioning and diffusion away from the surface into the PDMS bulk. Although LogP is a reasonable measure of how well a chemical partitions from aqueous solution into PDMS,⁵⁴ this partitioning is only at equilibrium near the interface. As has been shown previously, larger sequestration in PDMS-based microfluidics is associated with larger chemical diffusivity in PDMS,⁴⁴ and diffusion through PDMS membranes is slower for chemicals with a larger number of H-bond donors.⁵⁵ It is thus insightful, but not surprising, that the number of H-bond donors in a molecule can affect its sequestration by PDMS.

As shown in Fig. 4, one notable exception to the above trend is rhodamine 6G. This compound has two H-bond donor groups, and yet Wang *et al.* concluded that it bound PDMS strongly.⁴² When we tested rhodamine 6G in our experimental setup, we found a conflicting result with little to no PDMS binding. Both setups were depletion experiments, i.e., measuring the amount of chemical left in bulk solution after some duration of exposure to PDMS, but the experiments differed in the method used to measure chemical concentration. Our experiments used UV-Vis absorption, whereas Wang *et al.* used fluorescence intensity. Fluorescence is more sensitive, but also subject to photobleaching or quenching, which could explain the discrepancy by yielding an apparent depletion of rhodamine 6G even in the absence of PDMS binding. We thus consider absorption

spectroscopy a more robust measure of chemical concentration. Of note, rhodamine 6G was the only compound that Wang *et al.* quantified via fluorescence; the others were measured via radiolabels that are not subject to the same complications.⁴²

Beyond elucidating the molecular properties that correlate with PDMS binding, our experiments quantify binding to PDMS in a way that provides new insights. First, for three of the five PDMS-binding chemicals tested here, the carrying capacity of PDMS exceeded 1000 molecules per nm². Such carrying capacities are obviously much too large to represent pure surface packing and it is well known that small molecules can diffuse into the PDMS bulk.⁵⁶ Building on the model presented by Shirure and George,⁴⁴ one would expect the carrying capacity to increase with a chemical's diffusivity within PDMS. Such diffusivity is however difficult to measure directly for non-fluorescent molecules. Carrying capacity thus provides an alternative and more easily measurable parameter for bioavailability modelling that is valid at least over tens to hundreds of hours. This time regime is longer than the measured time constants associated with binding and desorption, which ranged from 2 to 18 hours.

These time constants are in a range that complicates the evaluation of multi-day chemical screening for targeted and/or adverse responses in microfluidically cultured cells and tissue constructs. Based on our modelling, the complications are three-fold. First, even if the nominal inlet concentration is constant, cellular exposure to a drug or potential toxicant will be time-dependent. Furthermore, the time needed to reach a steady-state exposure will be longer for lower inlet concentrations. Second, for chemicals that bind PDMS reversibly, even the steady-state cellular exposure will be less than the nominal dose – an order of magnitude less given the values we observe for the example of ethofumesate. Third, the delivery of acute doses of reversibly binding chemicals will be complicated by long tails of extended low-dose exposure long after a bolus injection. Our modelling approach shows that these complications can be estimated and thus considered in evaluating cellular responses. It may also be possible to use this modelling approach in a reverse manner to design a time-dependent inlet concentration profile that yields a targeted time-dependent cellular exposure.

Conclusions

We have established a technique to measure chemicals' PDMS-binding kinetics and a method to use these measured kinetic parameters to model chemical transport in PDMS-based devices and thus predict time-dependent cellular exposures. Further, we have found that binding to PDMS is not only correlated with measures of hydrophobicity such as LogP or TPSA, but also increases for compounds with fewer hydrogen-bond donor groups. This finding can serve as an exclusion criterion for compounds likely to have strong interactions with PDMS and thus difficult to interpret effects on cells in PDMS-based devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Wikswa JP, Exp. Biol. Med., 2014, 239, 1061–1072.
- Saadin K, Burke JM, Patel NP, Zubajlo RE and White IM, Microdevices, 2013, 15, 645–655.
- Konar D, Devarasetty M, Yildiz DV, Atala A and Murphy SV, Biomed. Eng. Comput. Biol., 2016, 7, 17–27. [PubMed: 27127414]
- Domansky K, Inman W, Serdy J, Dash A, Lim MH and Griffith LG, Lab Chip, 2010, 10, 51–58. [PubMed: 20024050]
- Bhatia SN and Ingber DE, Nat. Biotechnol., 2014, 32, 760. [PubMed: 25093883]
- Wikswa JP, Curtis EL, Eagleton ZE, Evans BC, Kole A, Hofmeister LH and Matloff WJ, Lab Chip, 2013, 13, 3496–3511. [PubMed: 23828456]
- Jo BH, Van Lerberghe LM, Motsegood KM and Beebe DJ, J. Microelectromech. Syst., 2009, 9, 76–81.
- Yeung T, Georges LA, Flanagan B, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V and Janmey PA, Cell Motil. Cytoskeleton., 2005, 60, 24–34.
- Murphy WL, McDevitt TC and Engler AJ, Nat Mater, 2014, 13, 547–557. [PubMed: 24845994]
- Moore TA, Brodersen P and Young EWK, Anal. Chem., 2017, 89, 11391–11398. [PubMed: 28972783]
- Martin MT, Knudsen TB, Reif DM, Houck KA, Judson RS, Kavlock RJ and Dix DJ, Biol. Reprod., 2011, 85, 327–339. [PubMed: 21565999]
- Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA and Fenner-Crisp PA, Crit. Rev. Toxicol., 2003, 33, 655–780. [PubMed: 14727734]
- D. HN and A. FP, J. Appl. Toxicol., 2011, 33, 265–272. [PubMed: 21935973]
- Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB and Al E., Toxicol. Sci., 2006, 90, 508–510.
- Yang C, Tan YS, Harkema JR and Haslam SZ, Reprod. Toxicol., 2009, 27, 299–306. [PubMed: 19013232]
- Macon MB, Villanueva LR, Tatum-Gibbs K, Zehr RD, Strynar MJ, Stanko JP, White SS, Helfant L and Fenton SE, Toxicol. Sci., 2011, 122, 134–145. [PubMed: 21482639]
- Dixon D, Reed CE, Moore AB, Gibbs-Flournoy EA, Hines EP, Wallace EA, Stanko JP, Lu Y, Jefferson WN, Newbold RR and Fenton SE, Reprod. Toxicol., 2012, 33, 506–512. [PubMed: 22146484]
- Biegel LB, Liu RC, Hurtt ME and Cook JC, Toxicol. Appl. Pharmacol., 1995, 134, 18–25. [PubMed: 7676454]
- Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C and Soto AM, Biol. Reprod., 2001, 65, 1215–1223. [PubMed: 11566746]
- Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubit BS and Al E., Reprod. Toxicol., 2008, 26, 209–210.
- Bhattacharya P and Keating AF, Toxicol. Appl. Pharmacol., 2012, 261, 227–235. [PubMed: 22531813]
- Quignot N, Arnaud M, Robidel F, Lecomte A, Tournier M, Cren-Olive C and Barouki R, Reprod. Toxicol., 2012, 33, 339–352. [PubMed: 22285353]
- Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenberg JG, Walser-Kuntz DR and vom Saal FS, Reprod. Toxicol., 2007, 24, 199–224. [PubMed: 17683900]

24. Munoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C and Soto AM, *Endocrinology*, 2005, 146, 4138–4147. [PubMed: 15919749]
25. Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C and Soto AM, *Proc. Natl. Acad. Sci.*, 2012, 109, 8190 LP–8195. [PubMed: 22566636]
26. Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J and Lamartiniere CA, *Environ. Heal. Perspect.*, 2009, 117, 910–915.
27. Betancourt AM, Eltoum IA, Desmond RA, Russo J and Lamartiniere CA, *Environ. Health Perspect.*, 2010, 118, 1614–1619. [PubMed: 20675265]
28. Hilakivi-Clarke L, *Breast Cancer Res*, 2014, 16, 208. [PubMed: 25032259]
29. Pei R-J, Sato M, Yuri T, Danbara N, Nikaido Y and Tsubura A, *In Vivo*, 2003, 17, 349–357. [PubMed: 12929590]
30. Whitsett TG and Lamartiniere CA, *Expert Rev. Anticancer Ther.*, 2006, 6, 1699–1706. [PubMed: 17181483]
31. Fritz WA, Coward L, Wang J and Lamartiniere CA, *Carcinogenesis*, 1998, 19, 2151–2158. [PubMed: 9886571]
32. Lamartiniere CA, *J. Mammary Gland Biol. Neoplasia*, 2002, 7, 67–76. [PubMed: 12160087]
33. Tou JCL and Thompson LU, *Carcinogenesis*, 1999, 20, 1831–1835. [PubMed: 10469631]
34. Dieras V, *Oncology*, 1997, 11, 31–33.
35. Magut PKS, Das S, Fernand VE, Losso J, McDonough K, Naylor BM, Aggarwal S and Warner IM, *J. Am. Chem. Soc.*, 2013, 135, 15873–15879. [PubMed: 24059469]
36. Gupta RS and Dudani AK, *J. Cell. Physiol.*, 2018, 130, 321–327.
37. [Comptox.epa.gov](https://comptox.epa.gov), EPA Chemical Database.
38. Jewell WT, Hess RA and Miller MG, *Toxicol. Appl. Pharmacol.*, 1998, 149, 159–166. [PubMed: 9571984]
39. Zhu W, Dang Z, Qiu J, Lv C, Jia G, Li L and Zhou Z, *Chirality*, 2007, 19, 632–637. [PubMed: 17554775]
40. Toepke MW and Beebe DJ, *Lab Chip*, 2006, 6, 1484–1486. [PubMed: 17203151]
41. Markov DA, Lu JQ, Samson PC, Wikswo JP and McCawley LJ, *Lab Chip*, 2012, 12, 4560–4568. [PubMed: 22964798]
42. Wang JD, Douville NJ, Takayama S and ElSayed M, *Ann. Biomed. Eng.*, 2012, 40, 1862–1873. [PubMed: 22484830]
43. van Meer BJ, de Vries H, Firth KSA, van Weerd J, Tertoolen GJ, Karperien HBJ, Jonkheijm P, Denning C, Ijzerman AP and Mummery CL, *Biochem. Biophys. Res. Commun.*, 2017, 482, 323–328. [PubMed: 27856254]
44. Shirure VS and George SC, *Lab Chip*, 2017, 17, 681. [PubMed: 28102869]
45. www.t3db.ca, Toxin Exposome Database.
46. <http://sitem.herts.ac.uk/aeru/ppdb/en>, Pesticide Properties Database.
47. Rothwell JA, Day AJ and Morgan MRA, *J. Agric. Food Chem.*, 2005, 53, 4355–4360. [PubMed: 15913295]
48. Gao X and Chorover J, *Environ. Chem.*, 2012, 9, 148–157.
49. Squires TM, Messinger RJ and Manalis SR, *Nat. Biotechnol.*, 2008, 26, 417–426. [PubMed: 18392027]
50. Gervais T and Jensen KF, *Chem. Eng. Sci.*, 2006, 61, 1102–1121.
51. Byron G, Daniel C, Xiaoyi H, A. R. Pand Carla W, *J. Mol. Recognit.*, 1999, 12, 293–299. [PubMed: 10556877]
52. Lionello A, Josserand J, Jensen H and Girault HH, *Lab Chip*, 2005, 5, 1096–1103. [PubMed: 16175266]
53. Vijayendran RA, Ligler FS and Leckband DE, *Anal. Chem.*, 1999, 71, 5405–5412. [PubMed: 21662737]
54. DiFilippo EL and Eganhouse RP, *Environ. Sci. Technol.*, 2010, 44, 6917–6925. [PubMed: 20726511]

55. Plessis JD, Pugh WJ, Judefeind A and Hadgraft J, Eur. J. Pharm. Sci, 2002, 15, 63–69. [PubMed: 11803132]
56. Baltussen E, Sandra P, David F and Cramers C, J. Microcolumn Sep., 1999, 11, 737–747.

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Fig. 1.

PDMS-binding and desorption experiments with example spectra for ethofumesate. **(A-left)** UV-Vis spectra showing depletion of ethofumesate from bulk solution as it partitions into a PDMS disk floating in the cuvette. **(A-right)** UV-Vis spectra showing return of ethofumesate to bulk solution as it desorbs into fresh solvent from a pre-soaked PDMS disk. **(B)** Dimensions of the microfluidic channel used in channel-soak experiments.

Fig. 2.

Time-dependent depletion of selected chemicals from bulk aqueous solutions in PDMS disk-binding experiments. A/A_0 = fraction remaining. Results ordered via descending LogP (listed beside each chemical structure). Data points with different symbols indicate different sample replicates. Solid lines are best fits to an empirical description (Eqn. 1); dashed lines are fits to a microscopic model for binding kinetics (Eqn. 3b). Dotted lines show a normalized value of 1.0 for chemicals with no discernible depletion.

Fig. 3.

Time-dependent return of chemicals into bulk aqueous solution via desorption from previously-soaked PDMS disks. Different symbols denote different sample replicates. Solid lines are best fits to an empirical description (Eqn. 2); dashed lines are fits to a microscopic model of binding kinetics (Eqn. 3b). Concentration is normalized to the amount depleted from solution, and thus bound to the disk, in the previous soaking experiment ($\Delta A_1 = -44.4 \mu\text{M}$ for ethofumesate; $-53 \mu\text{M}$ for molinate).

Fig. 4.

Correlation of PDMS binding affinity (% Bound) with chemicals' LogP and number of H-bond donors (subscript). Shaded region represents the LogP threshold for significant chemical absorption. (A) Disk-soak experiments. (B) Channel-soak experiments reported in this work (■), in van Meer *et al.*³⁹(○), or in Wang *et al.*³⁸ (△). Data points connected with horizontal lines denote discrepancies in reported LogP values.

Fig. 5.

CFD model predictions for continuous dosing with inlet concentrations from 10^{-7} to 10^{-2}

M: (A-C) predicted cellular exposures as a fraction of inlet exposures; (D-F) predicted degree of PDMS surface saturation. Chemical classes represented by ethofumesate with strong reversible binding, propiconazole with strong irreversible binding, and rhodamine B with weak reversible binding. The number next to each curve is log of the inlet concentration.

Fig. 6.

CFD model predictions for 4-h bolus dosing with inlet concentrations from 10^{-7} to 10^{-2} M: (A-C) predicted cellular exposures as a fraction of inlet exposures; (D-F) predicted degree of PDMS surface saturation. The number next to each curve is log of the inlet concentration.

Table 1

Commercial use and toxicity references for chemicals tested here.

Chemical Name	Use	Toxicity in Mammals	Ref.
perfluorooctanoic acid (PFOA)	Teflon™ manufacturing	endocrine disruptor	11–18
bisphenol A	plastic manufacturing	endocrine disruptor	11,19–27
diethylstilbestrol	synthetic non-steroidal estrogen	endocrine disruptor	28
genistein	pharmaceutical/supplement	endocrine disruptor	29–32
secoisolariciresinol diglucoside (SDG)	pharmaceutical/supplement	non-toxic	33
doxorubicin	chemotherapy	cytotoxic	34
docetaxel	chemotherapy	cytotoxic	34
rhodamine B or 6G	chemical dye	cytotoxic	35,36
propiconazole	fungicide	reproductive	11
aminopyralid	herbicide	developmental	37
molinate	herbicide	reproductive	11,38
ethofumesate	herbicide	non-toxic	39
imazaquin	herbicide	non-toxic	37
hexazinone	herbicide	reproductive	37
foramsulfuron	herbicide	non-toxic	37
sulfentrazone	herbicide	reproductive	11
acetamiprid	insecticide	reproductive	11
formetanate	insecticide	neurotoxin	37

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Summary of key molecular properties, experimental details and results for all chemicals tested: N = number of sample replicates; A₀ = initial chemical concentration; f_{B,eq}, A₁, τ₁, A₂, τ₂ from empirical fits to Eqn. 1 and 2 as defined in main text. Experiments not conducted and parameters that could not be estimated marked by dashes. The PDMS-surface-to-solution-volume ratio α is noted for each class of experiments.

Table 2.

Molecular Properties			Disk-Soak Experiments ($\alpha = S/V = 0.3 \text{ cm}^{-1}$)					Channel-Soak Experiments ($\alpha = S/V = 116 \text{ cm}^{-1}$)							
			Expt. Detail			Empirical Fit Parameters		Expt. Detail			Empirical Fit Parameters				
Chemical Name	LogP	TPSA ($\text{\AA}^2$)	H-bond donors	N	A ₀ (μM)	$f_{B,eq} = \frac{A_1}{A_0}$	τ_1 (h)	A ₂ /A ₀	τ_2 (h)	N	A ₀ (μM)	$f_{B,eq} = \frac{A_1}{A_0}$	τ_1 (h)	A ₂ /A ₀	τ_2 (h)
PFOA	6.3	37.3	1	5	589	26±4%	--	--	--	-	--	--	--	--	--
rhodamine 6G	5.2	59.9	2	3	20	1.7±0.3%	--	--	--	5	189	8±3%	--	--	--
diethylstilbestrol	5.07	40.5	2	3	89	4±3%	--	--	--	-	--	--	--	--	--
propiconazole	3.72	49.2	0	9	336	90±2%	9.7±1	--	--	-	--	--	--	--	--
bisphenol A	3.32	40.5	2	3	97	8±2%	17.6±1	--	--	3	488, 3100	78±1%	3.12±0.03	11.8±2%	0.2±0.2
genistein	3.04	87.0	3	3	38	1±1%	--	--	--	3	38	4±4%	--	--	--
molinate	3.21	45.6	0	3	113	50±10%	13.6±2	12±2%	6.1±1	-	--	--	--	--	--
ethofumesate	2.7	70.2	0	9	75	59±4%	11.3±1	19±1%	11.0±2	-	--	--	--	--	--
docetaxel	2.40	224.0	5	3	12	-0.1±0.8%	--	--	--	-	--	--	--	--	--
rhodamine B	1.95	52.8	1	3	10	2.05±0.05	--	--	--	3	177	80±5%	2.6±0.7	5±2%	1.5±0.8
imazaquin	1.86	91.6	2	3	17	-1.0±0.4%	--	--	--	3	16	13%	3.5±0.8	--	--
hexazinone	1.85	56.2	0	3	40	1.1±0.6%	--	--	--	3	40	37±7%	2.8±0.6	--	--
doxorubicin	1.27	206	6	3	60	6±1%	--	--	--	2	60	5±8%	--	--	--
sulfentrazone	0.99	90.5	1	3	26	-1.1±0.6%	--	--	--	2	26	-8.00±0.02%	--	--	--
acetamiprid	0.8	52.3	0	3	45	0.1±0.1%	--	--	--	2	45	-1±8%	--	--	--
formetanate	0	53.9	2	3	39	-2±3%	--	--	--	2	39	-9±3%	--	--	--
foramsulfuron	-0.78	177.0	3	3	32	-0.8±0.2%	--	--	--	2	32	7±3%	--	--	--
aminopyralid	-2.87	76.2	2	3	50	-2±1%	--	--	--	3	50	-14±7%	--	--	--
SDG	-2.93	258	10	3	58	0±3%	--	--	--	2	58	11±9%	--	--	--

Table 3.

Summary of microscopic model fit parameters

Chemical	k_F ($10^{-4} \text{ h}^{-1} \mu\text{M}^{-1}$)	k_R (10^{-2} h^{-1})	S_0 (nm^{-2})
propiconazole	3.7 ± 0.7	0	7300 ± 600
bisphenol A	0.3 ± 0.2	4.6 ± 0.7	500 ± 300
molinate	5 ± 1	0.9 ± 0.2	2500 ± 300
ethofumesate	5 ± 2	2.27 ± 0.4	2000 ± 1000
rhodamine B	3.2 ± 0.3	0.003 ± 0.002	8.0 ± 0.3
imazaquin	1.5 ± 0.5	0	0.13 ± 0.03
hexazinone	7 ± 2	0	0.7 ± 0.1