PDMS absorption of small molecules and consequences in microfluidic applications

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Microfluidic devices made out of polydimethylsiloxane (PDMS) have many physical properties that are useful for cell culture applications, such as transparency and gas permeability. Another distinct characteristic of PDMS is its ability to absorb hydrophobic small molecules. Partitioning of molecules into PDMS can significantly change solution concentrations and could potentially alter experimental outcomes. Herein we discuss PDMS absorption and its potential impact on microfluidic experiments.

Microfluidic devices have found use in cell culture applications due to the unique physical conditions that can be achieved within small channels. Precise patterning of cells, control over the cellular and subcellular microenvironments, and gentler cell handling have all been demonstrated using microfluidic devices. Ease of patterning via soft lithography, facile sealing, and optical transparency have led a large number of researchers to use polydimethylsiloxane (PDMS) to fabricate their microfluidic devices.

The network polymer structure of PDMS makes it highly permeable relative to other materials, a property that can be put to use in cell culture applications to supply oxygen and remove carbon dioxide. Diffusion of water vapor through PDMS has also been observed, which may be useful for some applications, but could lead to unwanted drying and convective flow within sample volumes. The porous nature of PDMS also enables small molecules to diffuse into the bulk polymer, which has allowed PDMS to be used in microextraction applications for removing trace organic compounds from aqueous samples. Silicone implanted into dogs was found to absorb the equivalent of 0.7% of its weight in small molecules, with lipids comprising approximately two-thirds of the absorbed mass. Similar uptake of small molecules by PDMS may affect the outcome of microfluidic experiments in drug discovery, proteomic analysis, and cell culture applications where species of interest are present in micro- and nanomolar concentrations.

Fig. 1 shows fluorescence images of a PDMS microfluidic channel after a series of filling steps with Nile red (MW 318 g mol⁻¹), a hydrophobic fluorophore. For the experiment, the channel was filled with 1 μM Nile red and incubated for one minute before the solution was aspirated out of the channel from the exit. Next, the channel was loaded with DI water, which was allowed to stand for one minute and then aspirated in preparation for the next sample volume. A fluorescence image of the channel after one filling with Nile red is shown in Fig. 1(a). The majority of the fluorescence appears as a halo around the inlet port of the channel, with essentially no signal from the main channel. The channel was filled in approximately one second, implying that absorption can occur rapidly given that little of the fluorophore reached the main channel. Figs. 1(b)–(e) show the fluorescence in the channel after several fills and rinsing with Alconox and DI water after the twentieth filling.

Fig. 1 Fluorescence images of Nile red absorbed into a PDMS channel after (a) one, (b) two, (c) six, (d) ten, (e) twenty fills, and (f) rinsing the channel with Alconox and DI water after the twentieth filling. (g) Cross-section and (h) substrate of PDMS channel used in (f). (i) A polystyrene substrate used with a PDMS channel of the same geometry and exposed to twenty fillings with Nile red and no rinse steps.

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subsequent filling steps (two, six, ten, and twenty). The fluorescence from the channel increased with each loading, indicating the accumulation of fluorophore. The same channel can be seen in Fig. 1(f) after rinsing with 200 channel volumes of an aqueous Alconox solution and 200 channel volumes of DI water; the negligible change in the fluorescence is indicative of the strong interaction of the dye with the polymer. A cross-section of the same PDMS device after the rinsing sequence (Fig. 1(g)) shows bright fluorescent signal more than 100 μm into the walls of the PDMS channel, revealing that the dye interaction with the PDMS is not limited to the surface of the channel. Fig. 1(h) and (i) show the top view of PDMS and polystyrene substrates that were exposed to 20 channel fillings with Nile red. The PDMS substrate shows a fluorescent signal after having been rinsed with Alconox and water. Although it was not rinsed after exposure to Nile red, the polystyrene substrate showed no visible fluorescence.

The absorption of small molecules into PDMS is an equilibrium process, analogous to liquid–liquid extraction, and therefore can be characterized by partition coefficients. Octanol/water partition coefficients are often used to predict PDMS absorption of small molecules for microextraction applications. Partition coefficients and kinetics for PDMS absorption of several pharmaceutical compounds, including ciprofloxacin and paclitaxel, have already been reported, and their values can vary greatly depending on factors such as pH and the ions in solution. The importance of pH and counterion pairing can make predicting PDMS absorption of small molecules difficult, especially for complex mixtures such as cell culture media.

The pH dependence of small molecule absorption is demonstrated in Fig. 2 with quinine (MW 324 g mol⁻¹), a drug that fluoresces at pH 2 but not pH 7. Fig. 2(a) shows the fluorescence image of a microfluidic channel filled with a pH 2 quinine solution (MW 324 g mol⁻¹). The fluorescent signal disappeared upon rinsing with pH 2 solution (Fig. 2(b)), indicating that little or no quinine was absorbed by the PDMS. Next, the channel was filled with a pH 7 quinine solution (Fig. 2(c)) and allowed to incubate for five minutes at room temperature. The channel was subsequently rinsed with pH 7 water and then filled with pH 2 water. A faint fluorescent signal was initially seen after the pH was changed from 7 to 2 (Fig. 2(d)) and the fluorescent intensity continued to increase over time until reaching equilibrium after five minutes (Fig. 2(e)). Finally, the channel was rinsed with pH 2 solution (Fig. 2(f)) and the fluorescent signal was lost, showing that essentially all of the quinine had repartitioned back into solution from the PDMS. The reversible nature of absorption shows that it is important to rinse channels out with medium prior to cell seeding if channels are preconditioned with any chemical treatment.

Absorbance of small molecules into PDMS microfluidic devices may have a profound effect on the outcome of drug screening studies, particularly when fixed volumes of drug are used to determine dose-response. Consider an assay performed with a hydrophobic pharmaceutical compound, such as quinine, in a PDMS channel that is 250 μm wide and 100 μm tall. A partition coefficient of 26 has previously been reported for microextraction of quinine from phosphate buffer solution (PBS) using PDMS fibers. If one were to try to hold the quinine concentration within the channel at 2 μM, the 100 μm-thick layer of PDMS surrounding the channel would have to absorb the equivalent of 100 channel volumes of 2 μM quinine. One channel volume of solution would contain 81 pg of quinine and the surrounding PDMS would be capable of absorbing 2 μg assuming 0.4 wt% small molecule uptake, indicating that the PDMS would have sufficient absorbance capacity. If the channel were only filled with 2 μM quinine solution one time, the solution concentration would be on the order of 20 nM, or 100 times lower than the intended concentration. Such a discrepancy would significantly alter the results of drug discovery screens, as apparent binding constants of compounds that interact strongly with PDMS would be significantly reduced.

Further reducing channel volumes with the aim of decreasing sample consumption will only further exacerbate the problem by increasing the surface area to volume ratio; for example, reducing the channel width to 125 μm would require the absorption of the equivalent of 160 channel volumes to achieve equilibrium between the PDMS and the solution in the channel. Paclitaxel, with a partition coefficient of 51, would need almost twice the excess required for quinine. While partitioning could be useful for sample collection/purification, such large excesses may negate any sample conservation that would be expected from performing assays in microfluidic devices. PDMS absorption, in addition to adsorption, has the potential to alter solution concentrations of cell culture components such as certain essential amino acids, vitamins, steroids, hormones, neurotransmitters, eicosanoids, and growth factors. The loss of such molecules could lead to reduced growth or alter phenotypic behavior in...
cell culture, which might then be incorrectly attributed to other factors.

Some steps can be taken to modify PDMS channels so that adsorption and absorption are reduced. Low molecular weight silicone molecules have been removed from bulk PDMS through aging and extraction, which reduced hydrophobic recovery after plasma treatment and increased solvent resistance. Excess crosslinking agent and zeolite filler have been incorporated into PDMS in order to reduce water evaporation. Unwanted adsorption of molecules onto the surface of PDMS can be reduced by first adsorbing BSA or by chemically modifying the PDMS surface with a silane. A method to coat the surface of PDMS channels with a thin polymer film by absorbing a photoinitiator into PDMS has been developed, but the technique has yet to be optimized for preventing absorption.

Ultimately, alternative fabrication materials or coatings may be required for microfluidic cell culture applications. A variety of different materials have already been developed for microfluidic applications; however, finding a material that meets the diverse set of requirements needed for cell culture could be difficult. Optical transparency, gas permeability, minimal investment in new equipment, and resistance to adsorption/absorption are just some of the desirable traits for new materials. A fluorinated polymer with properties similar to PDMS, but likely with fewer solute interactions, has been developed. Alternatively, microfluidic devices made out of materials that more closely mimic in vivo conditions are also being developed. While other materials may eventually be adopted, polystyrene has long been the material of choice for cell culture applications; however, prototyping polystyrene devices is both more difficult and expensive than soft lithography-based procedures. Therefore, PDMS can still play a key role in microfluidic device prototyping and characterization, but absorption should be taken into consideration because the composition of solutions may be significantly altered.

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