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Biocompatibility and Reduced Drug Absorption of Sol-Gel-Treated PDMS for Microfluidic Cell Culture Applications

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Abstract

Poly(dimethyl siloxane) (PDMS) -based microfluidic devices are now commonly used for a wide variety of biological experiments, including cell culture assays. However, the porous, hydrophobic polymer matrix of PDMS rapidly absorbs small hydrophobic molecules, including hormones and most small-molecule drugs. This makes it challenging to perform experiments that require such substances in PDMS microfluidic devices. This study presents evidence that a sol-gel treatment of PDMS that fills the polymer matrix with silica nanoparticles is effective at reducing the absorption of drugs into the material while preserving its biocompatibility, transparency, and oxygen permeability. We show that the absorption of two anti-cancer drugs, Camptothecin and a kinase inhibitor, is reduced to such an extent that on-chip microfluidic cell culture experiments can recapitulate the results obtained off-chip.

Poly(dimethyl siloxane) (PDMS) is a popular material for making microfluidic devices. This widespread utility stems from the compatibility of PDMS with both single- and multi-layer soft lithography^{1–3} as well as its high transparency, biocompatibility, low fluorescence, chemical inertness, and high gas permeability. Unfortunately, the very rapid absorption and diffusion of small hydrophobic molecules into PDMS limits its use in some biological applications, including cell culture experiments that require drugs with intracellular targets^{4–8}. In order for drugs to permeate the cell membrane and reach the interior of the cell, the molecules must be small (MW < ~500 Da) and hydrophobic, which are the same characteristics that favor diffusion into PDMS. It is, therefore, almost impossible to know and control the exact concentration of these drugs inside microfluidic channels when they are being lost into the channel walls. Methods that reliably reduce the diffusion of small molecules into PDMS will enable its use in a wide variety of biological and biochemical applications that involve small hydrophobic molecules in solution, such as toxicology profiling and high-throughput cell-based drug screening assays.

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Several methods to reduce nonspecific adsorption to and diffusion into PDMS in microfluidic devices have been reported⁹. The most common approach passivates the PDMS microchannel with either blocking proteins, such as bovine serum albumin, or detergents, such as *n*-dodecyl β -D-maltoside¹⁰ or pluronic¹¹. A second approach is the oxidation of the PDMS surface, followed by covalent attachment of hydrophilic molecules such as poly(ethylene glycol)¹² or epoxy-bearing molecules¹³. Another technique involves the deposition of multiple layers of polyelectrolytes of alternating polarities onto the PDMS surface^{14,15}. While these methods reduce the nonspecific adsorption of proteins onto the surface, they are very poor at preventing the nonspecific diffusion of small hydrophobic molecules into the material. High concentrations of organic solvent (e.g., 2–10% dimethylsulfoxide) could be added to the medium to favor partitioning of hydrophobic molecules into the liquid rather than the PDMS. This approach is compatible with some yeast species, but the solvent is itself absorbed by PDMS and toxic toward the majority of mammalian and other cell types. Furthermore, with this latter method it is still difficult to know and control the concentration of the drugs in the liquid. A recently reported technique uses sol-gel chemistry to coat the inside of PDMS microchannels with an 800 nm to 10 μ m thick layer of glass^{16,17}. This method results in virtually total inhibition of small molecule absorption into the PDMS and greatly increases the chemical resistance of the device. However, it is very hard to control the flow of the coating reagents through complex fluidic circuits with multiple inputs and outputs. Attempting to flow the sol-gel reagents through a complex fluidic circuit with integrated micro-valves results in the swelling of the valve membranes and loss of valve control. Additionally, this method is labor intensive and not amenable to batch fabrication of large numbers of devices.

Other polymers commonly used for making microfluidic devices, such as polystyrene, poly(methyl methacrylate), and polycarbonates, do not suffer from this absorption problem. These materials, however, are not suitable for soft-lithography due to their rigidity, and are not gas permeable, which complicates their use for most cell culture applications.

Recently, Roman *et al.* developed a simple sol-gel-based chemical treatment that permeates the PDMS with homogeneously distributed silica particles (~10 nm diameter)¹⁸. These particles fill the interstitial spaces in the polymer matrix and reduce the absorption of small hydrophobic molecules^{19,18}. The treatment consists of immersing the PDMS in tetraethyl orthosilicate (TEOS), which is rapidly absorbed by PDMS, followed by immersion in an organic base solution that catalyzes the condensation of TEOS into silica. The immersion time in TEOS determines the depth of TEOS diffusion into the PDMS and the extent to which the polymer matrix is filled with silica. This modification of the material is done *after* the microfluidic devices are fabricated by soft-lithography, so all the advantages of this fabrication procedure are preserved. Moreover, the treatment preserves the high gas permeability, chemical inertness, and optical transparency of PDMS, and can be easily performed on many devices at once.

In their initial work, Roman *et al.* demonstrated that the sol-gel treatment can substantially reduce the absorption and diffusion of the fluorescent dye rhodamine B. In this paper, we present results obtained from culturing human cells in microfluidic devices made of plain and sol-gel-treated PDMS, and exposing these cells to drugs that are rapidly absorbed by plain PDMS. These results indicate that sol-gel treatment preserves the biocompatibility of the material and drastically reduces the absorption of drugs used in cell biology experiments, with the level of absorption being dependent on the amount of silica that is loaded into the PDMS during treatment. At high enough silica loadings, drug absorption is reduced to such an extent that it is possible to perform microfluidic cell culture experiments using drugs that would be impossible to administer at well controlled concentrations in a plain PDMS device.

We also show that the diffusion of oxygen in the treated material is indistinguishable from that of plain PDMS.

EXPERIMENTAL SECTION

Materials and reagents

Unless otherwise noted, all reagents were purchased from Sigma-Aldrich (St. Louis, Missouri). All PDMS samples were made with RTV615 manufactured by Momentive Performance Materials (Albany, New York). Kelly neuroblastoma cells were kindly provided by Dr. William Weiss at the University of California San Francisco (UCSF) and cultured as described elsewhere²⁰. Camptothecin (Sigma) and the kinase inhibitor PW12 (synthesized according to published procedures²¹) were diluted at 10 mM in dimethylsulfoxide to create stock solutions that were kept refrigerated until use.

Sol-gel treatment

Sol-gel treatment starts by immersing the fully-fabricated PDMS chips (not mounted on glass) or blank pieces of PDMS in pure TEOS for the indicated times (*Caution! TEOS is a hazardous material. Appropriate procedures should be followed during handling and disposal*). The chips/pieces were constantly agitated during the first 5 min of the immersion to prevent them from sticking to the bottom of the glass container. After TEOS immersion, the PDMS was quickly rinsed with pure ethanol followed by de-ionized (DI) water, and immediately immersed in a 4% (v/v) solution of methylamine in DI water for at least 15 h. The ethanol rinse prevents the formation of silica crystals on the surface of the PDMS, which degrade the transparency of the material and interfere with its bonding to a glass slide. The chips were then rinsed thoroughly under running DI water. To ensure biocompatibility, some chips were immersed in DI water for at least 24 h (after the methylamine step), changing the water twice during the immersion, to remove the methylamine and any other contaminants from the interior of the PDMS. Finally, the PDMS was dried in an oven at 80°C to 95°C for at least 1 h.

Fluorescence and cell attachment assays

To obtain the fluorescein absorption and biocompatibility data as a function of TEOS immersion time (Figure 1a), cubes of PDMS (10 mm on each side) were treated with the sol-gel process described above, with and without the 24 h water immersion step. For cell attachment assays, the PDMS cubes were immersed in media (DMEM with 10% fetal bovine serum) containing Kelly cells at a concentration of 50,000 cells/ml. After 24 h, the cubes were washed twice with PBS and attached cells were counted under a microscope, with a 5x objective. Three separate fields of view were examined for each sample and averaged. For fluorescence assays, the PDMS cubes were immersed in a concentrated solution of fluorescein disodium salt (100 mM in PBS, pH 7.4) for 8 h. Following treatment, each sample was extracted for 30 min three times, with 3 ml of methanol each time, and the fluorescence intensity of the combined extracts was determined by spectrophotometry. Fluorescence intensities were normalized relative to the untreated PDMS sample.

To measure mass change as a function of TEOS immersion time (Figure 1b), pieces of PDMS (14 mm × 14 mm × 4 mm) were weighted before and after sol-gel treatment. One piece was used for each TEOS immersion of 5, 10, 20, 25, 40, and 50 min, while 4 pieces were used for a 15 min immersion, and 3 pieces for 30 and 60 min immersions.

Microfluidic device fabrication

All microfluidic devices were fabricated by soft lithography on silicon wafer molds made by standard photolithographic techniques (mold and chip fabrication have been described

elsewhere¹¹). The thickness of all the devices was between 4 and 6 mm (excluding the glass).

Valve-less chips were made by combining RTV615 cross-linker and elastomer at a 10:1 weight ratio, followed by mixing and de-bubbling in an automated mixer (5 min mix, 3 min de-bubble, AR-250, THINKY, Japan). The mixture was poured over the mold, which had been exposed to tetramethylchlorosilane vapors for 1 min, to a thickness of approximately 5 mm, de-gassed in vacuum, and baked at 80°C for one hour to cure. After curing, the PDMS was peeled off the mold, diced into individual chips, and input/output holes were punched with a 23 gauge coring catheter punch (Technical Innovations Inc., Brazoria, Texas).

Valved microfluidic devices were made using a design and fabrication protocol described elsewhere¹¹, with some protocol modifications. Instead of directly bonding the chips to a glass slide, the bottom of the devices was sealed with a plain PDMS membrane ~0.3 mm thick (made of 10:1 RTV615 cast on a plain silicon wafer) as follows: The chips and the fully cured membrane (still on the plain wafer) were rinsed with ethanol, dried by blowing nitrogen, treated with air plasma for 15 s at 70 W, 300 mTorr (Anatech SP-100, Anatech USA, Union City, California), brought into contact, and finally baked at 80°C for at least 20 min. After baking, the membrane was cut all around the chip and the bonded stack was peeled off the membrane-holding wafer.

To optimize the biocompatibility of the PDMS, all chips were baked at 80°C for at least 36 h after fabrication. The chips that received sol-gel treatment were treated after this long bake, and before they were mounted onto glass slides. The edges of the chips were slightly curled and swollen by the absorption of TEOS, so approximately 2 mm were trimmed from all four sides of the chips after the sol-gel treatment, to make them flatter and easier to bond to glass slides. Microscope glass slides for mounting the chips were cleaned with de-ionized water and Micro-90 cleaner (International Products Corp., Burlington, New Jersey), followed by exposure to air plasma for 180 s (115 W, 300 mTorr). Before bonding the chips to the glass, both plain and sol-gel chips were cleaned with adhesive tape, rinsed with ethanol, dried by blowing nitrogen, and exposed to air plasma for 15 s (70 W, 300 mTorr). The chips were then placed over the clean glass slides and baked at 80°C for at least 20 min for plain PDMS chips, and at least 1 h for sol-gel chips (overnight baking was preferred for sol-gel chips). During baking, the sol-gel chips were clamped between a 0.32 cm thick glass plate (in contact with the slide) and a 2.54 cm thick plastic block (in contact with the PDMS, with a roughened surface to prevent sticking), using large paper binding clips to apply force. Clamping was necessary to counteract stiffness and slight curling of the chips resulting from the sol-gel treatment.

Automated on-chip cell culture

All automated cell culture experiments were performed using an instrument and protocols described elsewhere¹¹. The growth medium used was RPMI with 10% FBS. At the beginning of each experiment, Pluronic F-127 (0.2 % w/w in PBS, filter-sterilized) was incubated for 1 h at 37°C inside the entire network of flow channels except the culture chambers, to passivate the PDMS surfaces. A sterile solution of human fibronectin (Chemicon International Inc., Temecula, California, 25 µg/ml in DPBS) was incubated in the culture chambers for at least 1 h at 37°C, to promote cell adhesion, and then rinsed with growth medium. Kelly cells for each experiment were obtained from either a fresh culture or a cryopreserved suspension. Fresh cultured cells were grown to just below confluency in a T25 flask, trypsinized, centrifuged, counted, and re-suspended in fresh medium at a concentration of $\sim 6 \times 10^5$ cells/ml. Cryopreserved cells were thawed in a water bath at 37°C, centrifuged, and resuspended in fresh medium to a concentration of $\sim 6 \times 10^5$ cells/ml. An

automated cell seeding protocol described elsewhere¹¹ was used to load approximately 80 cells in each culture chamber.

During culture, the chips were maintained at 37°C in a humidified atmosphere with 5% CO₂. Every hour, ~36 to 64% of the volume of each chamber was replaced with fresh medium, and the cells were imaged in phase contrast with 10x and 20x objectives. For the experiment shown in Figure 2a, the cells started receiving the drugs one hour after being seeded. For the experiments shown in Figure 2b and 2c all chambers received plain medium during the first 20 and 48 h of incubation, respectively. At the 21st and 49th hours, the full volume of all chambers was fully replaced with either plain medium, or medium with the drugs, depending on the experimental condition assigned to each chamber. At the end of the experiment shown in Figure 2b, the cells were fixed with paraformaldehyde (4% in PBS, incubated for 30 min, rinsed with PBS), stained with DAPI (1:1000 dilution in PBS, incubated for 7 min, rinsed with PBS), imaged, and counted automatically to determine the final population in each chamber.

As a control for each on-chip experiment, cells were simultaneously cultured in a 12-well culture plate (maintained in a humidified incubator at 37°C, with 5% CO₂) and manually received the drugs in the same schedule as the on-chip cells. These off-chip cultures were manually monitored for cell death 20 h after the administration of the drugs.

Manual on-chip cell culture

All manual on-chip cell culture experiments used DMEM with 10% FBS as the growth medium. A sterile solution of human fibronectin (Chemicon, 25 µg/ml in PBS) was incubated in the culture channels for at least 1 h at 37°C and then rinsed with growth medium. Crypreserved human foreskin fibroblasts (ATCC cat# SCRC-1041, designation HFF-1) were thawed in a water bath at 37°C, centrifuged, resuspended in fresh medium to a concentration of $\sim 4 \times 10^7$ cells/ml, and manually injected into the microfluidic channels using a pressurized pipet tip directly connected to the chip.

During culture, the chips were maintained at 37°C in a humidified atmosphere with 5% CO₂. Every hour, fresh medium was automatically injected into the channels at a very low flow rate, for 8 s. The channels were imaged manually at irregular intervals, in phase contrast, with a 10x objective. Off-chip cultures were used as a control for each on-chip experiment, as described in the preceding sub-section.

Oxygen diffusion measurements

Four slabs of PDMS (approx. 20 mm × 15 mm × 5 mm) were fabricated, with three of them receiving sol-gel treatment with TEOS immersions of 15, 30, and 60 min, and one remaining untreated. A 0.6 mm diameter hole was punched in the middle of each slab, to within approximately 0.5 mm of the bottom, and each slab was plasma-bonded to a glass slide. To create the oxygen sensor, the tip of an optical fiber-based oxygen meter (Microx TX3, PreSens Precision Sensing GmbH, Regensburg, Germany) was bonded inside a 0.65 mm diameter stainless steel tube using epoxy adhesive, with the sensing tip of the fiber protruding from the end of the tube by a few tens of micrometers. This steel tube was inserted as far as possible into the hole punched into each PDMS sample (creating a tight seal around the hole), and the sample plus the temperature probe of the oxygen meter were placed inside a container fitted with a gas inlet and outlet. After flushing the container with atmospheric air for at least 10 min and ensuring that the oxygen concentration inside the PDMS was equilibrated with the air, the gas flow was switched to pure nitrogen and the reduction in the oxygen concentration was recorded for at least 5 h. Time constants of the

decay were obtained from linear fits to the natural logarithm of the concentration data, for all data points between 1 h and 3 h after the switch to nitrogen.

RESULTS AND DISCUSSION

Initial characterization of sol-gel-treated PDMS involved measuring the absorption of fluorescein into the material, the proliferation of cells on its surface, and its increase in weight as a function of TEOS immersion time. Figure 1a shows that the amount of fluorescein absorbed by the material decreases dramatically with TEOS immersion times of 5 min or more (circle data points). An additional figure (Figure S-1) and a video of the absorption of fluorescein into the material can be found in the Supporting Information online. At the same time, cells proliferated normally on plain PDMS, as expected given the common use of PDMS microfluidic devices for cell culture¹¹, but as Figure 1a indicates, PDMS treated with the sol-gel process as reported by Roman *et al.*¹⁸ was toxic to the cells when the TEOS immersion was longer than 10 min (diamond data points). Under the hypothesis that the sol-gel treatment introduces unknown toxic contaminants into the PDMS, we tested immersing the material in ultra-pure water for at least 24 h after the silica condensation reaction, with several changes of the water during this time. With this water immersion step added to the treatment procedure, we saw no difference in biocompatibility between plain and sol-gel PDMS, for TEOS immersions of up to 60 min (Figure 1a, triangle data points). Additionally, Figure 1b shows a linear increase in PDMS mass, relative to the untreated material, as a function of TEOS immersion time, up to the 60 min immersion tested.

To investigate the use of sol-gel PDMS for real cell culture experiments using drugs that are rapidly absorbed by untreated PDMS, we performed a series of tests using an automated microfluidic cell culture system described elsewhere¹¹. This system is built around a PDMS device with 96 cell culture chambers, made using multi-layer soft lithography^{1,2}. We cultured cells from a human neuroblastoma line, referred to as Kelly cells²⁰, in chips made of plain and sol-gel PDMS, and exposed the cells to different concentrations of two drugs: PW12 and Camptothecin. PW12 (MW~400 Da) is an inhibitor of the p110 kinase (PI3K family) with potent antiproliferative activity²¹, while Camptothecin (MW~348 Da) is an inhibitor of DNA topoisomerase I used commonly as an anticancer agent²². As a readout of the cellular response to the drugs we used the cell motility measured over time from phase contrast images, as described elsewhere¹¹. In three experiments, the cells were seeded into plain and sol-gel treated PDMS chips and exposed to PW12 at 1 μ M and 4 μ M (plus 8 μ M in the plain PDMS chip), and Camptothecin at 10 μ M (only in sol-gel PDMS chips, as a positive cytotoxicity control). In conventional culture plates, these concentrations of the drugs lead to >90% cell death within 20 h of their administration (see Figures S-2 and S-3, SI online).

As Figure 2a illustrates, the motility of all the cells that were being administered the drug in a plain PDMS chip was not significantly different from that of cells not exposed to it, and we did not observe any cell death or significant changes to cell morphology during the course of the incubation. This is a clear indication that plain PDMS absorbed such a large fraction of the drug from the culture medium that it reduced its concentration well below the toxic level, even when PW12 was administered at 8 μ M. In this case, the reduction must be larger than 8-fold, since we know that PW12 at 1 μ M is lethal under conventional cell culture conditions.

Although Figure 1 demonstrates that a 2.5 min immersion in TEOS significantly reduces the absorption of fluorescein, Figure 2b shows that this length of treatment is not effective at reducing the absorption of drugs in cell culture experiments. Cells exposed to PW12 were

still not greatly affected by the drug, exhibiting a motility that is only marginally lower than that of cells not exposed to the drug. Camptothecin was lethal to the cells after approximately 48 h, compared to 20 h off-chip, and cells exposed to it were still able to proliferate for at least 24 h, indicating that this drug was also rapidly absorbed by the PDMS. In fact, Camptothecin diffused into the PDMS to such an extent that it clearly affected cells in chambers not receiving any drug, but that were immediately adjacent to those receiving it (data not shown).

With a 10 min TEOS immersion, drug absorption into sol-gel PDMS is reduced, as demonstrated by Figure 2c–d, although it is still substantial enough to prevent replication of off-chip results. In this case, PW12 was lethal after a ~100 h exposure at 1 μ M, and after 48 h at 4 μ M, while Camptothecin produced results similar to those in Figure 2b (>90% mortality after 48 h).

In the experiment shown in Figure 2b, DAPI staining was used to measure the proliferation in each chamber (see Figure S-4), and the final motility scores correlated very well with the proliferation of each condition, as Table 1 indicates (e.g. the lower the final motility, the lower the proliferation; see Figure S-5). In subsequent experiments, lethality was established by visual confirmation that approximately 90% or more of the cells were dead, which corresponded to a motility score of ~0.5. After the cells died, there was significant residual motility due to the dead cells detaching from the chamber floor and moving in response to the medium flow (motility scores between ~0.5 and ~0.25). Over time, this residual motility decreased as all the loose cells were washed out, reaching a minimum value of ~0.25. This minimum, non-zero motility is caused by the jitter of the microscope stage and small changes in illumination and focus between images. Figure 3b shows phase contrast images of the cells in representative chambers during the experiment from which the motility data on Figure 2c–d was extracted, further demonstrating the strong correlation between motility scores and cell health inferred from cell morphology. These images and the data in Table 1 demonstrate that motility scores, derived from phase contrast time-lapse images, are a reliable indicator of cell health, and can be a simple and very useful metric for studying cell reactions to drugs and other stimuli, without the need for fluorescence staining or other complex procedures. On the top row of the figure, cells cultured in plain medium (RPMI) appear healthy and proliferate during the whole duration of the experiment. A large number of cells exposed to Camptothecin (second row) look rounded and dead at 24 h, and most cells look dead or dying by 48 h. This is consistent with the sharp decrease in cell motility observed at 24 h. The morphology of cells exposed to the two concentrations of PW12 (two bottom rows) clearly shows how the cells start dying faster with PW12 at 4 μ M than at 1 μ M. At the end of the experiment, all cells exposed to the drugs were dead, as is evident in these images, and as indicated by motility scores lower than ~0.5 in Fig. 2d.

Sol-gel PDMS is stiffer than the untreated material, as Roman *et al.* have shown,¹⁸ and stiffness increases with increasing silica loading (longer immersions in TEOS). The closing pressures of microfluidic valves in a 5 mm thick sol-gel PDMS chip treated for 5 min in TEOS were between 23% and 50% higher than the pressures measured before treatment, in general agreement with the predictions of Roman *et al.*¹⁸

Treatment times of more than 10 minutes lead to a significant increase in valve closing pressures (>207 kPa or 30 psi), resulting in leaks and pressure-driven disconnections of control lines. A chip design with larger valve dimensions would be required to perform automated experiments in chips with high silica content. Consequently, all cell culture experiments done on sol-gel PDMS treated in TEOS for longer than 10 min were performed in valve-less chips with simple microfluidic channels, and monitored manually. For these experiments, we cultured human fibroblasts in microchannels made with plain and sol-gel

treated PDMS with a 60 min TEOS immersion. As Figure 4a shows, cells grown for 18 h on the sol-gel PDMS chip appear healthy, with morphology identical to that of the cells grown in the untreated PDMS device (Figure 4b). This demonstrates that sol-gel PDMS is biocompatible and amenable to cell culture applications for TEOS treatments up to 60 min (maximum time tested), as long as the material is thoroughly cleaned by water immersion after the silica condensation reaction, as mentioned earlier. Furthermore, injection of PW12 at a concentration of 2 μM resulted in complete cell death 20 h after the drug injection, consistent with the results obtained in conventional culture plates. Considering that PW12 at 4 μM in a sol-gel chip treated for 10 min in TEOS took ~48 h to kill the cells, this indicates that the 60 min TEOS immersion dramatically reduced drug absorption into the material.

Finally, it should be noted that the gas permeability of sol-gel PDMS remains high, even after a 60 min TEOS immersion. We observed that dead-ended microfluidic channels can be filled with liquid as easily as in plain PDMS, indicating that air can diffuse into the polymer at approximately the same rate in both cases. Possible changes in oxygen permeability were quantitatively assessed by measuring the diffusion of oxygen, as a function of time, out of an untreated slab of PDMS, and sol-gel treated ones with TEOS immersions of 15, 30, and 60 min. The slabs were initially equilibrated with atmospheric air, followed by exposure to pure nitrogen starting at time zero, resulting in the diffusion of oxygen out of the material. As Figure 5 shows, the time constants for the decay in oxygen concentration are very similar across all four samples: 0.68 hrs (untreated), 0.70 hrs (15 min TEOS), 0.65 hrs (30 min TEOS), and 0.70 hrs (60 min TEOS). Evidently, there is no significant reduction in oxygen permeability with sol-gel treatment up to 60 min of TEOS immersion.

CONCLUSIONS

In summary, we have demonstrated that a sol-gel treatment of PDMS, which fills the polymer matrix with silica nanoparticles, is effective at blocking the absorption of biologically relevant drugs into the PDMS while preserving the bio-compatibility, oxygen permeability, and transparency of the material. At the same time, the treatment process is compatible with device fabrication by single- and multi-layer soft lithography, and can be performed on many devices at once (e.g. batch processing). Bio-compatibility is ensured by thoroughly cleaning the treated PDMS by immersion in DI water for at least 24 h, to remove contaminants introduced by the sol-gel process. Without this cleaning step the material is harmful to cells for TEOS immersions longer than 10 min. Using this treatment protocol, we have shown that two anti-cancer drugs, Camptothecin and kinase inhibitor PW12, which are quickly absorbed by plain PDMS, can be successfully used in cell culture experiments inside sol-gel treated PDMS microfluidic devices that have a large enough silica loading (TEOS immersion for 1 h). These experiments replicated off-chip results, indicating that drug absorption into sol-gel PDMS is negligible. Given that the two drugs used in this study have sizes and levels of hydrophobicity typical of the molecules that are rapidly absorbed by PDMS, we expect that the sol-gel treatment will be equally effective against the absorption of a wide range of small hydrophobic molecules. Additionally, we have shown that for TEOS immersions of up to 1 h, the transport of oxygen through sol-gel PDMS is virtually identical to that of plain PDMS.

Further work is required to obtain more detailed and quantitative measurements of the extent of drug absorption into sol-gel PDMS to fully understand the limitations of the treatment. Additional work will also be needed to quantify the impact of the increased stiffness of sol-gel PDMS on the design rules for valved microfluidic devices created by multi-layer soft lithography²³.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Basic characterization of the sol-gel treatment. **(a)** Fluorescein absorption (circles) and biocompatibility (diamonds and triangles) of sol-gel treated PDMS, as a function of TEOS immersion time, relative to untreated PDMS. Triangle data points correspond to samples that were cleaned by water immersion after treatment, while diamond points correspond to samples that were not cleaned. All data are relative to untreated PDMS. **(b)** Increase in PDMS mass as a function of TEOS immersion time, relative to the untreated material. Error bars indicate the standard deviation derived from replicate measurements at three immersion times (15, 30, and 60 min).

Figure 2.

Cell motility as a function of time, extracted from three separate experiments with Kelly cells cultured on-chip in either plain growth medium (RPMI) or one of two different cytotoxic drugs: Camptothecin injected at 10 μ M, and PW12 injected at 1 μ M and 4 μ M (plus 8 μ M in **a**), all diluted in growth medium. Time zero corresponds to the point where the drugs were injected into the chambers. **(a)** Plain PDMS chip, where drug absorption into the PDMS reduces PW12 concentrations below the cytotoxic level (no observable decrease in cell motility). No cytotoxic effects were observed even when PW12 was injected into the chip at 4 μ M. Each data point is an average of the motility extracted from two images. **(b)** Sol-gel treated PDMS chip with a 2.5 min immersion in TEOS. Drug absorption was still high enough to reduce the concentrations of PW12 below the cytotoxic level, even when injected into the chip at 4 μ M. The Camptothecin concentration remained high enough to be lethal after 48 h of exposure. **(c)** Sol-gel treated PDMS chip with a 10 min immersion in TEOS. Drug absorption was reduced enough to make PW12 cytotoxic only when injected at 4 μ M, but not at 1 μ M. The Camptothecin concentration remained high enough to be lethal

after 48 h of exposure. **(d)** Same as (c) but showing data up to 120 h, when all cells exposed to drugs died. In (b) through (d) each data point is an average of the motility extracted from three images, and the error bars indicate the standard error of the mean.

Figure 3.

Phase contrast images of the cells in representative chambers during the experiment from which the motility data on Figure 2c–d was extracted (10 min TEOS immersion). Each row corresponds to a particular condition, and each column to a time point. The drugs were injected at time 0 h.

Figure 4. Images of human fibroblasts cultured on-chip, in plain (right column) and sol-gel treated PDMS (left column) with TEOS immersion for 60 min and a 24 h water cleaning process, and exposed to kinase inhibitor PW12 injected at a concentration of 2 μ M in DMEM. Top row: 18 h after seeding, before injection of PW12. Bottom row: 45.5 h after seeding, 20.5 h after injection of PW12. **(a,b)** Growth of cells in plain and sol-gel treated PDMS is qualitatively indistinguishable, indicating that the sol-gel treatment did not affect the biocompatibility of the material. **(c,d)** PW12 absorption into the sol-gel PDMS was reduced enough to keep the concentration above the lethal level. At the same time, diffusion of the drug in untreated PDMS lowered the drug concentration sufficiently that cells appear healthy.

Figure 5. Oxygen concentration as a function of time inside slabs of PDMS with different levels of sol-gel treatment. The slabs were initially equilibrated with atmospheric air, followed by exposure to pure nitrogen starting at time zero, resulting in the diffusion of oxygen out of the material.

Table 1
Final motility and proliferation vs. experimental condition

Final average proliferation (expressed as final cell population relative to initial cell population) and motility score, plus/minus the standard error of the mean, for the different conditions in the experiment corresponding to Figure 2b (automated on-chip culture, 2.5 min TEOS immersion, see Figures S-4 and S-5 in the Supporting Information).

Condition	Proliferation	Final Motility
RPMI	10.02 ± 0.24	1.31 ± 0.01
Camptothecin 10 μM	2.85 ± 0.21	0.31 ± 0.01
PW12 1 μM	7.81 ± 0.29	1.19 ± 0.01
PW12 4 μM	6.80 ± 0.33	1.17 ± 0.03