Article

Evaluation of Surface Treatments of PDMS Microfluidic Devices for Improving Small-Molecule Recovery with Application to Monitoring Metabolites Secreted from Islets of Langerhans

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ABSTRACT: Microfluidic devices are becoming an important tool for bioanalysis with applications including studying cell secretion, cell growth, and drug delivery. Small molecules such as drugs, cell products, or nutrients may partition into polydimethylsiloxane (PDMS), a commonly used material for microfluidic devices, potentially leading to poor recovery or inaccurate delivery of such chemicals. To decrease small-molecule partitioning, surface and bulk PDMS treatments have been developed; however, these have been tested on few analytes, or their biocompatibility are unknown. Studies often focus on one analyte, whereas a diversity of chemicals are of interest and possibly affected. In this study, 11 device treatments are tested and applied to 21 biologically relevant small molecules with a variety of chemical structures. Device treatments are characterized using water contact angle measurements and evaluated by measuring recovery of the 21 target analytes using liquid chromatography—mass spectrometry. 1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene), a positively charged polymer, produced the least hydrophilic surface and was found to provide the best recovery with most of the analytes having >50% recovery and up to 92% recovery; however, recovery varied by analyte highlighting the importance of analyte diversity rather than targeting a single analyte in evaluating treatments. A polybrene-treated device was applied to investigate secretion from pancreatic islets, which are micro-organs involved in glucose homeostasis and diabetes. Islets secrete small molecules that have been shown to modulate the secretion of islets' main functional products, glucose-regulating hormones. The polybrene treatment enabled the detection of 20 target analytes from islets-on-chip during isosmotic and hypo-osmotic glucose perfusions and resulted in detection of more significant secretion changes compared to untreated PDMS.

KEYWORDS: microfluidics, liquid-chromatography, mass spectrometry, small molecules, metabolites, islets, surface treatment
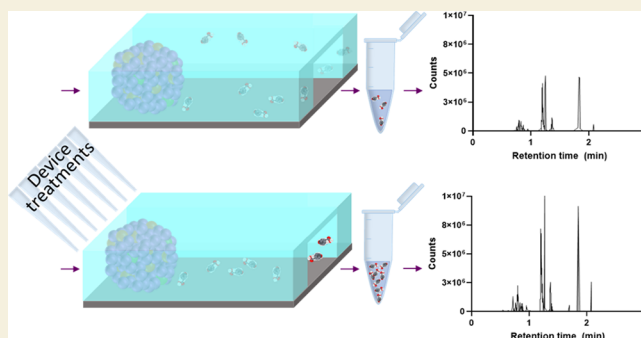
1. INTRODUCTION

Microfluidics has facilitated secretion measurements from many[1−4] or single[5,6] cells, studies on drug delivery,[7] cell—cell interaction investigations,[8,9] and on-chip cell culture.[10] Polydimethylsiloxane (PDMS) has become the most popular material for microfluidic-based studies of cells because it is safe and simple to use, elastomeric, biocompatible, and oxygen permeable.[11] A commonly cited advantage of microfluidic devices for biological applications is precise control of the cellular environment; however, small molecules adsorb to or partition into PDMS likely due to its porous and hydrophobic nature,[12,13] thus limiting control of the chemical environment. Chemical losses due to surface interactions affect the ability to recover molecules when monitoring cellular secretion, the potential for chemicals to diffuse to cells for cell—cell interaction, and the ability to dispense drugs or nutrients to cells. Given the widespread use of PDMS, it is valuable to elucidate mechanisms of analyte loss to PDMS and identify methods to minimize loss. Mitigating chemical partitioning will

enable more accurate drug delivery, better cell culture, and improved recovery of analytes.

Several surface and PDMS bulk modifications have been investigated to control molecular interactions with PDMS including treatment with oxygen plasma,[14] sol—gel,[15] silanization,[16] polyvinyl alcohol (PVA),[17] and surfactant[18] among others. Typically, studies of treatments have utilized a limited number of model chemicals to test the potential for reducing losses. For example, one study investigated the hydrophobicity changes of 13 PDMS treatments and assessed six of the treatments for recovery of Nile red.[12] Another study focused
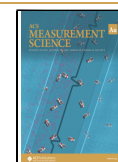
only on partitioning of estrogen from cell culture media into PDMS and the impact of such partitioning on on-chip cell culture.[19] Other investigations focused on the partitioning of small hydrophobic molecules into PDMS using only Nile red[20] or Rhodamine B[21] as model compounds. Direct studies of losses for a diversity of analytes are unavailable. Indirect observations, e.g., from band broadening of electrophoresis separations performed on PDMS devices,[22] suggest potential for surface interactions even for polar compounds, although quantification of losses has not been reported.

Studies of the mechanisms leading to chemical loss on PDMS suggest that multiple factors are involved. Increasing surface hydrophilicity is a common goal to improve small-molecule recovery that rests on the assumption of a hydrophobic interaction with the surface being important for retention.[17,20] However, increasing PDMS surface hydrophobicity, such as through parylenes and hydrophobic polymer coating, has also improved recovery of small hydrophobic molecules.[12,23] Porosity and surface topology have also been identified as potential contributing factors to chemical partitioning.[12] Here, we test a variety of treatments that include changing surface charge, hydrophobicity, and device porosity to further understand mechanisms of retention.

To evaluate the effectiveness of the best treatment on recovery of a variety of chemicals, we measured metabolite secretions from islets of Langerhans. Islets are micro-organs that contribute to glucose homeostasis and diabetes and secrete chemically diverse molecules ranging from hormones like insulin to small molecules. These secretions have been postulated to regulate or indicate their status with respect to metabolic control.[24] Increasing the recovery of these compounds from PDMS/glass microfluidic devices will facilitate studies aimed at elucidating mechanisms of secretion and cell−cell interaction. As part of this work, we investigate the effect of osmolarity on metabolite secretion since this has been suggested to affect secretion independent of glucose metabolism. For example, it has been proposed that taurine plays an osmoregulatory role in islets, and taurine and GABA have been found to have increased secretion during hypo-osmotic conditions.[25,26] A mechanism for nonvesicular release of GABA due to osmotic changes has been proposed and implicates the volume-regulated anion channel (VRAC), which controls the cell volume and opens under hypo-osmotic conditions, as the means of this secretion.[25] There is potential for other molecules to be released via this mechanism, or others, due to osmotic changes. However, there is a lack of information on other components of the islet secretome and how they respond to osmotic changes, which may provide more insight into islet regulation and function. Due to the regulatory role that small molecules can have on the hormonal output of islets, a better understanding of the regulation of secretion of small molecules is of interest.

2. METHODS

2.1. Chemicals and Reagents

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich (Saint Louis, MO). Sodium hydroxide (NaOH), HPLC-grade solvents, cell culture chemicals, 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (polybrene, PB) with a molecular weight of 374.2 (part number NC9840454), Gibco RPMI-1640 cell culture medium, and chemicals for making Hank's balanced salt solution (HBSS), sodium chloride, potassium chloride, magnesium sulfate heptahydrate, sodium bicarbonate, magnesium chloride hexahydrate,

and calcium chloride dihydrate, were purchased from Fisher Scientific (Waltham, MA). Sodium phosphate dibasic anhydrous was obtained from Acros Organics (Geel, Belgium). Isoflurane was purchased from MWI Animal Health (Boise, ID). Collagenase P was purchased from Roche Diagnostic (Indianapolis, IN). Nile red was purchased from Cayman Chemical (Ann Arbor, MI). Serotonin and PVA with a molecular weight between 88,000 and 97,000 (part number 41243) were obtained from Alfa Aesar (Ward Hill, MA). HBSS solutions were prepared in house with 18 MΩ water purified by a Series 1090 E-Pure system (Barnstead International, Dubuque, IA) and filtered with 0.2 μm nylon syringe filters (Fisher) prior to use.

2.2. Islet Isolation and Culture

Pancreatic islets were harvested from anesthetized CD-1 mice (Envigo, Indianapolis, IN) as described before.[27] Briefly, after collagenase P ductal injection, the pancreas was removed for digestion, washing, and filtering. Islets were handpicked into fresh RPMI-1640 cell culture media supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 unit/mL penicillin and stored at 37 °C, 10% CO_2, pH 7.4. All islets were used within 4 days of isolation and ranged in size between 100 and 150 μm. More details are provided in the Supporting Information. Animal procedures were approved by the University of Michigan Institutional Animal Care and Use Committee and conducted in Association for Assessment and Accreditation of Laboratory Animal Care-accredited laboratories.

2.3. Microfluidic Chip Fabrication, Design, and Operation

Microfluidic devices were designed using Autodesk, Inc. AutoCAD software (San Rafael, CA) and printed onto a transparency (Fineline Imaging, Colorado Springs, CO). To create master molds with a 25 μm feature height, an SU8 2025 negative epoxy photoresist was spin-coated onto silicon wafers (University Wafer, South Boston, MA) for 30 s at a spin speed of 3570 rpm. Ultraviolet irradiation polymerized the photoresist exposed through the transparency, and then, the photoresist was developed. PDMS devices were made using a degassed 1:10 activator-to-monomer ratio poured over the master mold. Devices were cured for at least 2 h at 70 °C before removal from the master mold and further curing for at least 2 h at 100 °C. All devices were then bonded to a glass slide using oxygen plasma activation (Harrick Plasma, Inc., Ithaca, NY). PDMS/glass devices were heated at 70 °C for at least 2 h before use.

A PDMS/glass device was used for islet-on-chip studies as previously described[1] (SI Figure S1). Briefly, perfusate was pumped through two inlets, a mixing channel and then an islet chamber to an outlet for fraction collection. The concentration of perfusate components can be modified by changing syringe-driven flow rates through the two inlets. The islet chamber was open to the atmosphere for loading of islets but afterward was plugged with a PDMS-filled capillary so that solution from the inlet flowed over the islets and out of the chip for fraction collection. After collection, samples were derivatized and analyzed by LC−MS as described below. The device was kept at 37 °C using a thin film resistive heater placed underneath the device during operation with islets.

For recovery testing, the microfluidic device was composed of only the islet chamber and outlet, including the outlet capillary, to mimic exposure of secreted analytes to the device and capillary during islet-on-chip experiments. Treatments were applied to both PDMS and outlet capillary. The channel post-islet chamber was 3 mm long, 25 μm tall, and 80 μm wide. Analytes were perfused through one inlet and collected in 30 min fractions from the outlet capillary. Unless otherwise noted, all perfusions were at 6 μL/min.

2.4. Treatments

2.4.1. Untreated PDMS and Capillary. Untreated devices were used following bonding with no alterations. For fused silica capillary experiments, which were used as a control, samples were perfused through the capillary and collected directly from untreated capillaries to assess recovery.

2.4.2. Water Soaking. A microfluidic device was fully submerged in water for 2 days and then immediately used.

2.4.3. Polybrene. A 1% PB (w/v) aqueous solution was perfused through the device at 3 μL/min for 5 min. After 15 min of statically resting PB in the device, the device was flushed with HBSS for 15 min at 6 μL/min.[28]

2.4.4. Sodium Dodecyl Sulfate. A 0.03% sodium dodecyl sulfate (SDS) in water (w/v) solution was perfused through the device at 5 μL/min for 45 min. Immediately following, the device was flushed with HBSS for 15 min at 6 μL/min.[29]

2.4.5. Layered PB and SDS. A 1% PB solution (w/v) was perfused through the device at 3 μL/min for 5 min. After 15 min of rest, a 0.03% SDS solution (w/v) was perfused at 3 μL/min for 5 min. After another 15 min of rest, the device was flushed with HBSS for 15 min at 6 μL/min.

2.4.6. 5% Bovine Serum Albumin Pretreatment. Devices were flushed sequentially with 100 μL of a 5% bovine serum albumin (BSA) solution (w/w), a wash of 100 μL of water, and then at least 50 μL of HBSS at 6 μL/min.[1]

2.4.7. 0.1% BSA Coflow. Standard mixes were made in HBSS containing 0.1% BSA (w/w). No device treatments were performed.

2.4.8. PVA. To make a 1% solution of PVA in water (w/w), solid PVA was added to a vial containing a stir bar. While stirring, ice-cold water was added dropwise. The total mass was recorded, and the mixture was stirred at room temperature for 40 min. The temperature was then increased to 100 °C and held for 40 min then decreased to 65 °C and stirred overnight. Evaporation was corrected for by adding water the following day.[17]

The PVA solution was added to the device until it was full. After 10 min at room temperature, nitrogen gas was used to purge the device of solution. The device was heated at 110 °C for 15 min before use.

2.4.9. PVA and Oxygen Plasma. A 1% PVA solution in water (w/w) was perfused through the channels immediately after bonding the device. After 10 min, devices were purged with nitrogen gas and heated in a 110 °C oven for at least 2 h before use.

2.4.10. Nile Red Saturation. A 100 μM Nile red in dimethyl sulfoxide (DMSO):ethanol (EtOH):water (0.01:1:3) solution was prepared by first dissolving Nile red in DMSO and then diluting in EtOH and water. The Nile red solution was perfused for 12 h at 0.6 μL/min.

2.4.11. Layered PB and Dextran Sulfate. NaOH (0.1 M) and water were sequentially flushed through the microfluidic device for 4 min each. Next, 1% PB (w/v) was perfused for 10 min followed by a 15 min incubation. Then, 3% dextran sulfate (DS) (w/v) with a molecular weight of >500,000 (part number S4030) was perfused for 2 min followed by a 15 min incubation.[30] All flow rates were 6 μL/min.

2.4.12. Trichloro(1H,1H,2H,2H-perfluorooctyl)silane and Oxygen Plasma. Immediately after oxygen plasma bonding devices, channels were perfused with 2% (v/v) trichloro(1H,1H,2H,2H-perfluorooctyl)silane (PFOCTS) in perfluorodecalin. The derivatizing solution was evaporated from the channels in a 110 °C oven for at least 2 h.

2.4.13. PDMS Slabs. For water contact angle (CA) measurements, PDMS slabs were used. To treat the PDMS slabs, the slabs were submerged in treatment solutions and shaken to emulate flow through a device. For incubations, the shaking was paused, and the slabs were allowed to statically rest in the solution. The treatment time and composition were kept identical to device treatments.

2.5. Device Recovery

Device recovery experiments were performed the same regardless of treatment. Immediately following device treatment if applicable, HBSS was perfused and collected as a blank sample from three devices. Then, a solution containing between 50 and 2500 nM of each analyte (detailed concentrations in Table S1) was perfused for 3 h, and fractions were collected every 30 min. Samples were immediately derivatized as described later. The original solutions for the amino acid and biogenic amine mixtures were also derivatized for recovery calculations. The recovery over 3 h was averaged to produce the overall recovery reported.

2.6. Surface Characterization

Water CAs were used to characterize the hydrophobicity of the surface of treated PDMS slabs from 2 h to 14 days after treatment. Measurements were made 2 h, 1 day, 2 days, 3 days, and 4 days after treatment and then every other day until day 14.

To make water CA measurements, 30 μL of water was deposited on the surface of a PDMS slab 6 replicate times. From each droplet, 3 replicate measurements were taken and quantitated using a goniometer (Ramé-Hart goniometer model 120-F0, Netcong, NJ) and DROPimage software (Ramé-Hart, Netcong, NJ). Measurements were taken at the same time of the day.

2.7. Islets-on-Chip

Seven different groups of 8 islets were used for these studies. Islets were from a total of 4 mice. Islets were washed in 3 mM glucose in HBSS before they were loaded on-chip where they were perfused at 6 μL/min. Islets were allowed to equilibrate for 30 min with perfusion of 3 mM glucose in HBSS. Islets were next perfused with 11 mM glucose, 1 mM glucose, and 11 mM glucose in HBSS for 20 min each. Mannitol was added to the HBSS to keep the osmolarity constant. Then, islets were perfused for 20 min with 1 mM glucose without mannitol added, causing a decrease in osmolarity. Fractions were collected into tubes every 2 min. Immediately, the samples were derivatized as described below and kept at −80 °C until they were used.

2.8. LC−MS and Small-Molecule Monitoring

Quantification of amino acids and biogenic amines was performed using a previously established LC−MS method.[1,31] Briefly, samples and standards were derivatized using benzoyl chloride (BzCl). To samples or standards, 100 mM sodium carbonate in water, 2% BzCl in acetonitrile (ACN), and internal standards (IS) in 80/20 (v/v) ACN/water with 1% sulfuric acid were added in a 2:1:1:1 ratio by volume (sample:carbonate:BzCl:IS). To prepare internal standards, ^{13}C-BzCl was used to derivatize a standard mix of amino acids and biogenic amines. After derivatization, samples were analyzed in triplicate using a Phenomenex (Torrance, CA) Kinetex C18 chromatography column (100 × 2.1 mm, 1.7 μm particles with 100 Å pores) on a Vanquish ultrahigh-performance liquid chromatography system (Thermo) interfaced to a TSQ Quantum Ultra triple-quadrupole mass spectrometer (Thermo). Automated peak integration was performed using XCalibur 3.0 MS software, and all peaks were visually inspected to ensure proper integration.

2.9. Statistics and Calculations

Statistical analyses used to determine the significance of differences in secretion between glucose perfusions were conducted using RStudio. For this longitudinal study with limited sample sizes, a mixed-effects model analysis was selected. A mixed-effects model allows for missing values by making efficient effect estimates, accounts for device or mouse heterogeneity through use of covariate assigned random effects, and has been shown to have more statistical power than ANOVA, especially with small sample sizes ($n < 8$).[32] Since our work aims to describe response over time, has some missing values (some data points from islets-on-chip secretions were below the limit of detection (LOD)), and uses a small sample set, a mixed-effects model is a favorable choice for these data.

For islet-on-chip significance comparisons, four groups were created: high glucose 1, low glucose, high glucose 2, and low glucose with a hypo-osmotic shift. Each group was compared to every other group for significance. For other significance testing outside of the islet-on-chip data, Student's *t*-tests were used. Significance was defined as $p < 0.05$ (*). Further significance was defined as $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

3. RESULTS AND DISCUSSION

3.1. Water CA of Devices after Surface Treatments

Hydrophobicity of PDMS after treatment was assessed using water CA (Figure 1). All recovery testing was done
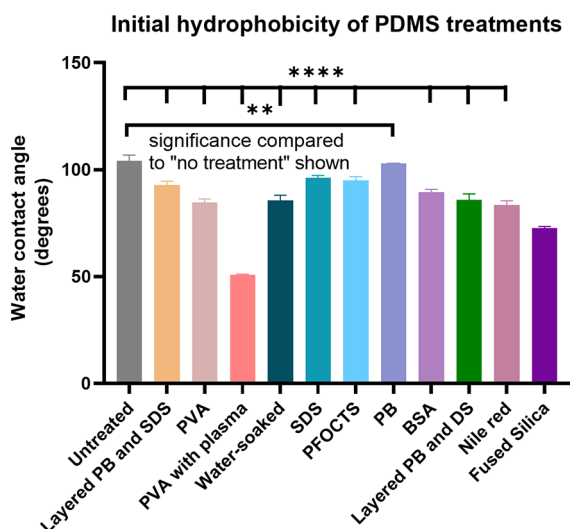
Figure 1. Water contact angle measurements to determine hydrophobicity of PDMS treatments. Water CA measurement 2 h after treatment, which is when islets would be loaded on-chip. Significance testing was done between untreated PDMS and each other treatment. The measurement associated with SDS was not significantly different, while PB was significantly lower. All other treatments were significantly different from untreated PDMS. Plotted as means $\pm$ 1 standard deviation (SD). Significance was identified by Student's t-test and defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). $N = 6$.

immediately following treatment, but we assessed the water CA from 2 h to 14 days after treatment to determine stability (SI Figure S2). In between stability measurements, PDMS slabs were stored in air. Most treatments were stable for 2 days or longer, though recovery testing was started within 2 h of treatment.

Untreated PDMS was used as a control. The CA of untreated PDMS was $104.2 \pm 2.4°$ (Figure 1), in agreement with other reports indicating CA > 100°.[33,34] No treatments increased the water CA, which would have indicated an increase in hydrophobicity. Two hours following treatment, the most hydrophilic treatment was PVA onto oxygen plasma-treated PDMS (PVA with plasma) with a water CA of $50.8 \pm 0.5°$. The next most hydrophilic treatments include Nile red, PVA, water soaking, and layered PB and DS with respective initial water CAs of 83.5 ± 1.9, 84.6 ± 1.6, 85.7 ± 2.3, and $86.0 \pm 2.7°$. PB was the most similar to untreated PDMS with an initial measurement of $102.9 \pm 0.2°$ followed by SDS, PFOCTS, layered PB and SDS, and BSA respectively measured as 96.1 ± 1.1, 94.9 ± 1.8, 92.9 ± 1.7, and $89.6 \pm 1.2°$. The measured water CAs of previously evaluated treatments agree with other studies in that the CA decreases with treatments including PVA,[17] water soaking,[35] and BSA.[36]

3.2. Screening of Device Treatments for Small-Molecule Recovery

We tested recovery of 21 amino acids and biogenic amines perfused through a PDMS/glass device following various surface treatments. Recovery was measured every 30 min for 3 h from the treated and untreated surfaces. For most analytes, recovery was consistent across the 3 h sampling period (SI Figure S3), so the average recovery across this period is reported. The one exception was Nile red, which showed a 10% increase in recovery over 3 h for most analytes (SI Figure S3).

Treatments chosen for testing are expected to be biocompatible at the concentrations present on-chip.[16,17,37] While SDS is known to lyse cells, the amount remaining on-chip after treatment is likely low enough to not cause detrimental effects; however, if treatments are found to be detrimental to cell health, treatments could be selectively applied post-cell chamber and re-evaluated. Selected treatments are also MS-compatible (i.e., do not wash out of the device enough to interfere with MS or are at low enough concentrations to not interfere).

We first evaluated analyte recovery after flowing through the fused silica capillary, which is present at the outlet of the chip (see SI Figure S1) to establish a baseline recovery possible in the system without PDMS. For the 21 target analytes, recovery from capillary ranged from 19 to 100% and averaged 68% (Figure 2). The recovery of some analytes was surprisingly low,
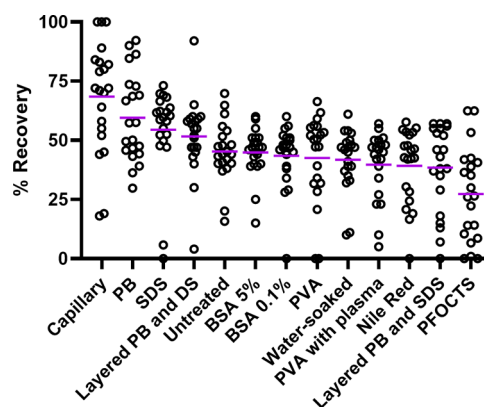

Figure 2. Recovery values and means by treatment. The % recovery for each analyte is plotted as a function of surface treatment. If an analyte was not detectable (i.e., below the LOD), it was marked as 0% recovery. Bars indicate the mean for all analytes for a given treatment. Each point represents the mean of 3 measurements per time point across 6 time points for 3 replicate devices, but error is not displayed for simplicity.

especially compared to previous work where samples were collected through similar capillaries.[38,39] We found that the carrier solvent, HBSS in this case, played a role as substantially higher recoveries were found with other physiological solutions like the artificial cerebral spinal fluid; nevertheless, we used the HBSS values as the baseline for comparisons to effects of treatments. Another factor contributing to lower than anticipated recovery is the loss of molecules to collection tubes. Molecule interaction with Eppendorf tubes contributed to the loss of glutamate and aspartate notably. For PDMS/glass, recovery ranged from 15 to 69% and averaged only 45%.

We tested 5% BSA solution for device pretreatment because we had previously found it to improve recovery of hormones from a PDMS/glass device;[1] however, this treatment had a poor effect on small-molecule recovery as recovery ranged from 15 to 60% and averaged 45%. We also tested 0.1% BSA in the experimental buffer[40,41] but found relatively poor recovery of small molecules with this method as recovery was 0 to 60% and averaged only 43%. BSA did not offer improvement when comparing recovery with and without treatment (Figure 3). While BSA treatment is effective for hormones, different treatments are needed for small molecules.

We tested PVA treatment, which increases hydrophilicity of surfaces,[17] based on the hypothesis that a more similar surface
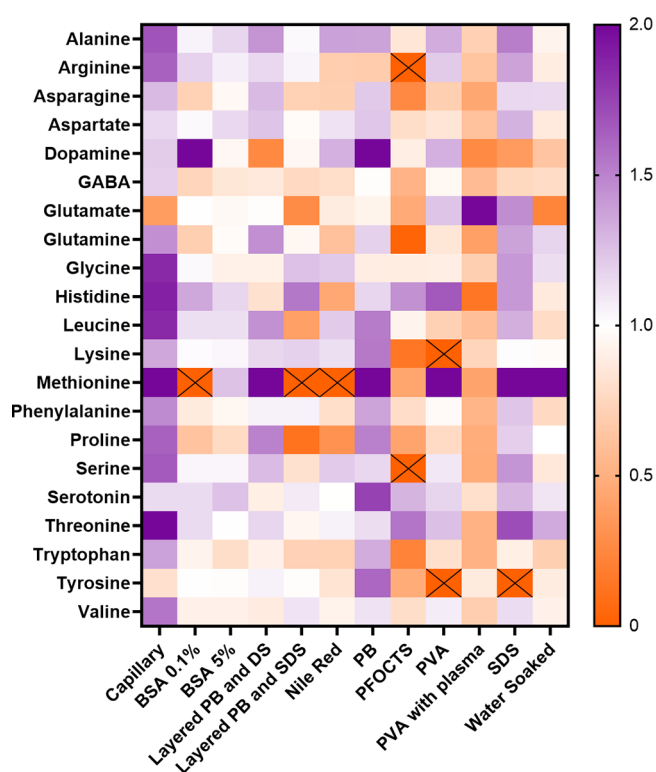
Figure 3. Improvement of analyte recovery compared to untreated PDMS. This heat map represents the recovery of each analyte for a given treatment compared to the recovery of that analyte from an untreated PDMS/glass device. If the ratio is >1, there is an improvement in recovery using that treatment. If no analyte was detected, it was represented as an x. Plotted as the mean, $n = 3$.

to perfusate would decrease the analyte loss. PVA was either deposited immediately after oxygen plasma treatment or several hours later. For PVA application hours after oxygen plasma treatment, which led to a slight increase in hydrophilicity, recovery for analytes was from 0 to 66% and averaged 43%. For PVA application immediately following oxygen plasma treatment, which produced a more hydrophilic surface, recovery for analytes was from 5 to 57% and averaged 40%. For both PVA treatments, analytes with hydrophobic side chains did not have improved recovery when compared to an untreated device (Figure 3). PVA treatments did not have the intended effect; thus, treatments aimed at changing other aspects of PDMS were explored.

PFOCTS was selected to produce a potentially non-interactive fluorocarbon coating. The PFOCTS treatment did not perform well as analyte recovery ranged from 0 to 62% and averaged 27%. PFOCTS treatment is not suitable for improving small-molecule recovery from PDMS.

PB was selected as a positively charged species[30] for treatment; however, we also found it to have a similar water CA to PDMS. On PB-treated surfaces, analytes had recovery from 30 to 92% with an average recovery of 60%, a result comparable to recovery observed from the capillary alone (Figure 2). Compared to no treatment, PB improved recovery for 2 of 3 positively charged analytes (Lys and His) and only slightly reduced recovery for 1 of 2 negatively charged analytes (Glu) (Figure 3), indicating that the improvement could not be easily linked to ionic interactions. Given the success of this treatment, a negatively charged surface treatment was also tested. SDS has been used for PDMS treatments with the

understanding that the hydrophobic tail partitions into the bulk of the PDMS, leaving the polar head group exposed to increase hydrophilicity and negative charge of the device.[29,42] SDS treatment resulted in recovery for analytes from 0 to 73% and averaging 54% (Figure 2). This surface treatment improved recovery for both negatively charged analytes (Asp and Glu) but interestingly did not decrease recovery of positively charged analytes (Figure 3). When examining recovery across all analytes, PB and SDS each improved recovery for different analytes. Given these results, layering the two ionic species was also tested. Layered PB and SDS had recovery for analytes from 0 to 57% with an average recovery of 38% (Figure 2). Layering was less successful than either treatment alone.

As charged surfaces gave an overall better analyte recovery (PB and SDS treatments), we tested a combination of positively charged PB and the negatively charged polymer, DS. This treatment has been used to reduce protein adsorption to a capillary[28] and has been applied to electrophoresis in a PDMS/glass device to achieve greater stability than for PB coating alone.[30] Layered PB and DS had recovery for analytes from 4 to 92% and averaging 52%, making it one of the best performing treatments.

PDMS is porous, which may allow accumulation of small analytes into pores and poor recovery; therefore, we tested treatments that had been reported to fill pores. Water soaking of PDMS has been shown to increase wettability of PDMS and change the surface roughness of fluidic channels.[35] It may fill openings in the device and decrease analyte accumulation into pores. However, water-soaked devices had recovery for analytes from 10 to 61% and averaging 42% (Figure 2). Nile red, a small hydrophobic molecule, which readily partitions into PDMS, was selected to saturate the bulk of the PDMS device in an attempt to decrease analyte partitioning.[12] However, this approach was also not successful at reducing the analyte loss. Nile red treatment resulted in recovery for analytes from 0 to 57% and averaging 39%. These data indicate that a change in surface roughness and decreased porosity do not greatly contribute to the analyte loss on-chip; however, materials that may fill pores and prevent partitioning may still be desirable.

As no PDMS treatment yielded high recovery for all of the tested analytes, it is possible to evaluate recovery using several criteria (Figure 2). When considering the ability to detect all analytes, the range of recovery, and the average recovery, PB, SDS, and layered PB with DS come the closest to fused silica, showing that they most effectively negate the loss to PDMS. PB treatment matches or exceeds the layered PB and DS treatment in these categories. Further, PB treatment shows high recoveries for half of the target analytes and recoveries around 50% for the rest, while for other treatments, recoveries for certain analytes are only at ∼50% or less. When assessing recovery relative to an untreated device (Figure 3), PB treatment performed better than layered PB and DS or other treatments with only 3 analytes (Glu, Gly, and Arg) having worse recovery than untreated PDMS. Other treatments had 4 to 14 analytes with worse recovery. While SDS improved recovery for as many analytes as PB (16), PB overall outperformed SDS because the PB treatment enabled the detection of all analytes (i.e., all analytes were above their LOD) and a greater average recovery of all analytes than SDS. The PB treatment also allowed for recovery up to 92% as opposed to only 73% for the SDS treatment. PB treatment was

thus selected for islet-on-chip experiments; however, it is important to measure recovery for analytes of interest. If Arg were of special interest, PB would not be the best choice (Figure 4). Likewise, SDS outperforms PB for 11 analytes
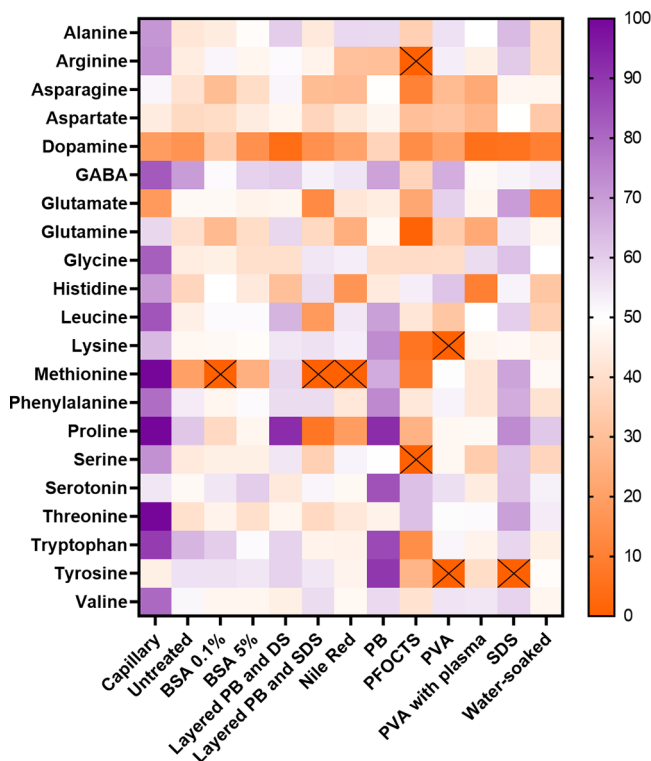

Figure 4. Recovery of 21 target analytes for each PDMS treatment. This heat map represents the recovery from not detectable (x) or 0 to 100% of analytes perfused for 3 h through either a capillary, an untreated device and a capillary, or a treated device and a capillary. The capillary alone has the best overall recovery followed by PB and SDS. Plotted as the mean, $n = 3$.

(Figure 4) highlighting the significance of analyzing the recovery of target molecules for a given system and of studies targeting more than one analyte when evaluating treatments.

The treatment with the best overall recovery of analytes, PB, had the closest hydrophobicity to untreated PDMS. These and previously published data[12,43] suggest that hydrophobic interactions may not be the strongest determiner of analyte retention on-chip.

This work confirms that small molecules are readily lost to PDMS but also demonstrates that the loss varies widely by analyte. It is challenging to find a surface treatment that is suitable for a wide range of analytes. Such effects may be especially important in cell culture studies where a variety of chemicals may be important in growing or differentiating cells, including many of the analytes targeted in this study. When culturing cells on-chip, additives are lost to the PDMS bulk, which decreases the effective available amino acids for cells. Further, the loss likely varies as the surface area to volume of a device changes, further altering what compounds are available to cells. There has been a push to characterize the drug loss to PDMS devices before conducting experiments with the drugs;[13,15] likewise, there should be a push to better understand the environment of cells during on-chip cell culture.

3.3. Monitoring Small Molecules from Islets-on-Chip

We next used PB-treated PDMS chips to perfuse islets and collect fractions to determine secretion patterns for the 21 analytes (Figures 5 and 6). Our objective was to test this
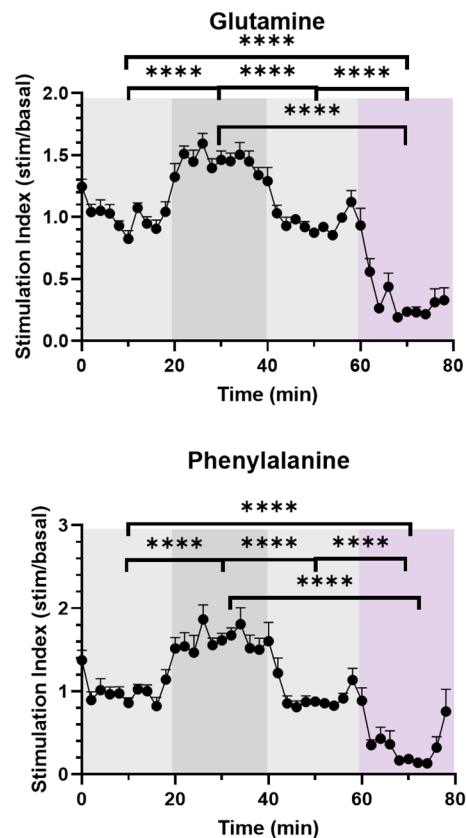

Figure 5. Amino acids from islets-on-chip with a decrease in secretion when glucose and osmolarity decrease but an increase in secretion when only glucose decreases and osmolarity is fixed. After equilibration on-chip, 8 islets were exposed to 11 (light gray) or 1 (dark gray) mM glucose without a change in osmolarity for 20 min each. The last 20 min is a 1 mM glucose perfusion and a hypo-osmotic shift (purple). Significance is plotted, and each 20 min period was compared to each other 20 min period for significance determination using a mixed-effects model. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Plotted as means $\pm$ 1 SEM. $N = 7$ groups of islets collected from 4 mice.

system by distinguishing metabolite secretion associated with glucose stimulation from secretion evoked by osmolarity change. For this experiment, the glucose concentration in HBSS was changed from 11 to 1 to 11 mM every 20 min during the first hour. Osmolarity was kept constant through addition of mannitol when glucose decreased. After this hour, a hypo-osmotic shift was induced by eliminating mannitol and decreasing glucose from 11 to 1 mM in HBSS. This pattern allows comparisons to be made when changing from 11 to 1 mM glucose under isosmotic and hypo-osmotic conditions. It also allows comparisons of osmolarity effects independent of glucose by comparing 1 mM glucose with and without mannitol. For statistical analysis, every 20 min period was compared to every other 20 min period.

Two analytes, Phe and Gln, were secreted significantly more by shifting from 11 to 1 mM glucose perfusion when
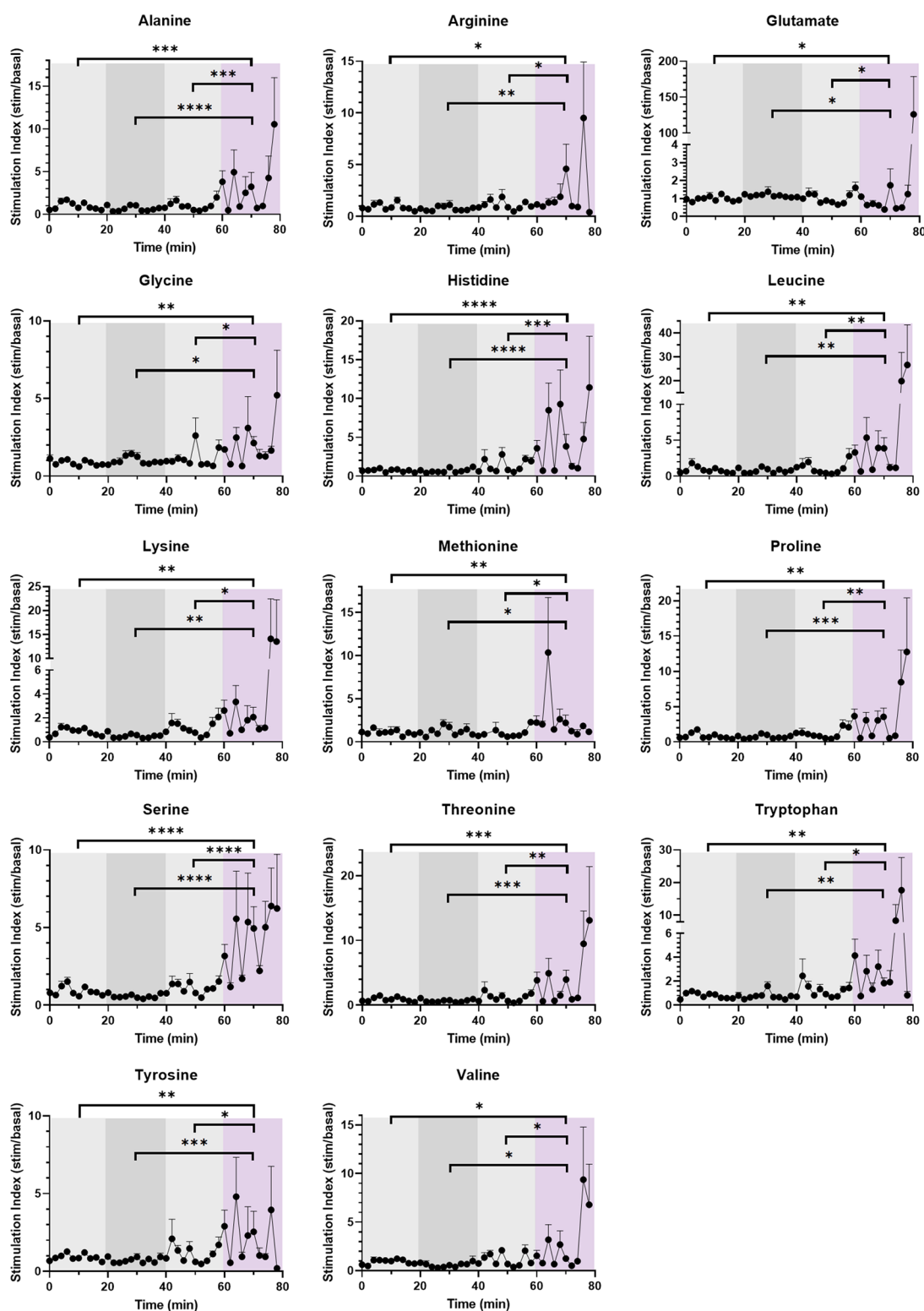
Figure 6. Amino acids from islets-on-chip with an increase in secretion when glucose and osmolarity decrease. After equilibration on-chip, 8 islets were exposed to 11 (light gray) or 1 (dark gray) mM glucose without a change in osmolarity for 20 min each. The last 20 min is a 1 mM glucose perfusion and a hypo-osmotic shift (purple). Significance is plotted, and each 20 min period was compared to each other 20 min period for significance determination using a mixed-effects model. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Plotted as means ± 1 SEM. $N = 7$ groups of islets collected from 4 mice.

osmolarity was held constant, suggesting that glucose metabolism plays a role in regulating their release to the extracellular space (Figure 5). No other metabolite was significantly affected by isosmotic glucose changes.

When allowing osmolarity to change with glucose, 16 analytes (Trp, Phe, Leu, Tyr, Lys, Val, Met, Pro, Ala, Thr, Glu, Gly, Ser, Gln, Arg, and His) had a significant change in secretion (Figure 6). For Phe and Gln, secretion decreased as

glucose decreased inducing a hypo-osmotic shift. Phe and Gln were altered by glucose metabolism as well, showing that these two amino acids are regulated by both processes. For the 14 remaining analytes, secretion increased with decreasing glucose and the hypo-osmotic shift, corresponding to other reports for all[44] or some (Val, Ser, Arg, and His)[45] (Glu)[46] small molecules. All of these compounds also had significantly more secretion with low glucose after a hypo-osmotic shift compared to low glucose with maintained osmolarity. These results indicate that osmolarity governs the release of these compounds. Unlike the change with metabolism for Gln and Phe, the changes with these compounds tended to be "noisy" with fluctuations between high and low levels of secretion that nevertheless resulted in an overall significant increase at low glucose without mannitol. The "noisy" appearance and the error associated with peaks of secretion can be mainly attributed to temporal differences in short bursts of secretion form different clusters of islets tested (e.g., SI Figure S4).

Asp and Asn, contrary to another report,[44] did not have a significant change in secretion upon decreasing glucose and changing osmolarity; however, Asp and Asn were released significantly more when both glucose and osmolarity changed than when glucose alone changed (SI Figure S5).

When comparing each 20 min period, DA and GABA did not exhibit statistically significant secretion changes between glucose levels or osmolarities (SI Figure S6). The result for GABA is surprising because a previous study reported an increase in GABA secretion during a hypo-osmotic shift,[25] and we detected statistically significant differences with glucose uncorrected by osmolarity. That report used a BSA-treated PDMS device.[1] However, one other report has shown no significant changes for GABA release with decreasing glucose and osmolarity, agreeing with this result.[44] When examining individual replicates from this work for significance, significant differences in secretion occur for 4 of 7 replicates. We previously observed variability in GABA response in which some islet sets released pulses of GABA and others had more sustained secretion.[1] This variability in secretion may impact the power of measurements; e.g., in Figure S6, we observe that some data points for GABA are elevated in the 1 mM glucose period but with high variability due to differences in timing of secretion thus precluding significance. It is also possible that hormones are involved in mediating the release, and the BSA treatment used previously was more effective at maintaining hormone concentrations.

In summary, Phe and Gln secretion increased with decreasing glucose and fixed osmolarity but decreased with decreasing glucose and decreasing osmolarity. These were the only metabolites measured that appeared to have secretion controlled by glucose metabolism. Changes in osmolarity affected many amino acids, presumably via VRAC channels.[25] Secretion significantly increased for 14 analytes (Trp, Leu, Tyr, Lys, Val, Met, Pro, Ala, Thr, Glu, Gly, Ser, Arg, and His) with decreasing glucose and decreasing osmolarity. Asp and Asn were released significantly more when both glucose and osmolarity were changing as opposed to only glucose changing. DA and GABA had no significant differences observed between 20 min periods.

Importantly, dynamics that were not previously identified have been illuminated with the improved recovery of amines from PDMS. When changing glucose from 11 to 1 mM without fixed osmolarity, 16 analytes have significant changes in this study compared to only 4 detected previously using this

system with BSA-treated PDMS.[1] Recovery for all but 1 of these analytes improved (13 analytes) or did not change (2 analytes) with PB treatment as opposed to the 5% BSA treatment previously used,[1] likely contributing to discerning more significant secretion changes.

4. CONCLUSIONS

Hydrophobicity of PDMS does not appear to play a dominant role in small-molecule recovery from PDMS/glass devices. Charge-based PDMS treatments that maintain the hydrophobicity, measured by the water CA, of untreated PDMS hold promise as PB and SDS treatments gave two of the best performances for small-molecule recovery, though layered PB and DS also performed well with a slight decrease in hydrophobicity. Testing treatments on a broader range of analytes than is typical demonstrated the importance of targeting more than one analyte, especially when seeking to apply studies to cell secretions or cell culture. Applying a treatment favorable for recovery of most small molecules for the islets-on-chip study revealed changes in secretion during osmotically balanced and unbalanced perfusions that had not been previously observed on similar PDMS devices. Since changes in osmolarity mimic physiological conditions and islet secretions change, further studies should be conducted to better understand these changes and islet function.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmeasuresciau.3c00025.

> Islet isolation details, analyte abbreviations and recovery testing concentrations, microfluidic device figures, stability trends of device treatments, recovery over time, demonstration of variability in temporal response of islets-on-chip, and secretion results for asparagine, aspartate, dopamine, and GABA from islets-on-chip (PDF)

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