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Amphiphilic silicones to reduce the absorption of small hydrophobic molecules

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ABSTRACT

Silicones (i.e. crosslinked poly(dimethylsiloxane), PDMS) are commonly used material for microfluidic device fabrication. Nonetheless, due to the uncontrollable absorption of small hydrophobic molecules (<1 kDa) into the bulk, its applicability to cell-based drug assays and sensing applications has been limited. Here, we demonstrate the use of substrates made of silicones bulk modified with a poly(ethylene oxide) silane amphiphile (PEO-SA) to reduce hydrophobic small molecule sequestration for cell-based assays. Modified silicone substrates were generated with concentrations of 2 wt.%, 9 wt.% and, 14 wt.% PEO-SA. Incorporation of PEO-SA into the silicone bulk was assessed by FTIR analysis in addition to water contact angle analysis to evaluate surface hydrophobicity. Cell toxicity, absorption of small hydrophobic drugs, and cell response to hydrophobic molecules were also evaluated. Results showed that the incorporation of the PEO-SA into the silicone led to a reduction in water contact angle from 114° to as low as 16° that was stable for at least three months. The modified silicones showed viability values above 85% for NIH-3T3, MCF7, MDA-MB-468, and MDA-MB-231 cell lines. A drug response assay using tamoxifen and the MCF7 cell line showed full recovery of cell toxicity response when exposed to PDMS modified with 9 wt.% or 14 wt.% PEO-SA compared to tissue culture plastic. Therefore, our study supports the use of PEO-SA at concentrations of 9 wt.% or higher for enhanced surface wettability and reduced absorption of small hydrophobic molecules in PDMS-based platforms.

Statement of significance

Silicones, such as poly(dimethylsiloxane) known as PDMS, are commonly used material for microfluidic device fabrication, yet the uncontrollable absorption of small hydrophobic molecules (<1 kDa) into the bulk of silicones, limit their applicability into drug assays and hydrophobic sensing applications in aqueous solutions. The present study examined the hydrophilic properties and cell compatibility of PDMS combined with poly(ethylene oxide) silane amphiphile (PEO-SA). The data shown supports the use of PEO-SA at concentrations of 9 wt.% or higher for enhanced and stable surface wettability and reduced absorption of small hydrophobic molecules in silicons for cell-based and hydrophobic sampling applications.

1. Introduction

Poly(dimethylsiloxane) (PDMS) is frequently cross-linked to form hydrophobic silicone elastomers. Silicones have many attractive physical, chemical and mechanical properties [1,2] for micro- and nano-scale fabrication and molecular assay prototyping including easy fabrication, gas and vapor permeability [3], non-toxicity [4], good elasticity [5], high chemical resistance [6], thermal and oxidative stability [7], low modulus [8], irreversible bond to different materials [9], low-cost, and optical transparency [4,10]. Currently, silicones play a major role in cell-based applications, being preferred by engineers for the fabrication of microscale and

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nanoscale fluidic devices [5] employed in molecular assays and cell culture platforms ranging from surface micropatterning to the casting of channels and 3D geometries [1].

Despite these advantages, the silicone-based microdevices have been shown to produce non-desirable culture artifacts for cell-based assays and sensing applications wherein hydrophobic compounds are examined. This stems from the absorption of biomolecules such as plasma proteins (e.g. fibrinogen) and other small hydrophobic molecules (<1 kDa) sequestered from the culture media (e.g. steroid hormones and drugs) [1,11]. The absorption of hydrophobic molecules has been shown to shift observed drug potency and influence cell behavior. In this regard, several methods have been evaluated to reduce the surface hydrophobicity of silicones, including physical, chemical and physico-chemical modification [12,13]. However, due to silicone's low surface energy and high chain flexibility [14] the hydrophilicity gained is not permanent and hydrophobic recovery occurs within hours or days depending on the modification employed [15]. Chemical modification methods include plasma grafting, chemical coating, surface-modifying additives (SMAs), UV-generated ozone, and oxygen plasma [16,17]. While plasma treatment is simple, efforts to stop hydrophobic recovery such as cold storage at −80 °C are not practical [18]. Several approaches have combined plasma treatment with surface polymer grafting, or surface-deposited coating layers (i.e. physisorption), but involve complex processes, and hydrophilicity is often not retained [12,13,19]. For instance, silicone surfaces have been treated with polyvinylpyrrolidone (PVP) [20], poly (acrylic acid) [21–23], poly(vinyl alcohol) [24–26] or poly(ethylene oxide) (PEO) [19,27]. PEO is of particular interest as it is biocompatible [28] and is known for its antifouling properties stemming from steric repulsion mechanism, blockage of adsorption, and repulsive hydration [29,30]. Surface-grafting of PEO onto model substrates (e.g. glass, silica) has been widely utilized to demonstrate enhanced surface hydrophilicity and decreased adsorption of proteins [31–33].

The use of PEO-based SMAs represents a simple and potentially potent approach to increase the hydrophilicity of silicones via bulk modification. Such a system would require that the PEO chains migrate rapidly and substantially to the aqueous/biological interface to effect surface hydrophilicity. However, hydrophobic recovery was observed for both condensation cure (i.e. RTV) silicones bulk-modified with conventional PEO silanes (e.g. triethoxysilyl-propyl PEO monomethyl ether, $(EtO)_3$-Si-$(CH_2)_3$-PEO_n-OCH_3) [34,35] and for addition cure silicones bulk-modified with allyl PEO monomethyl ether (CH_2=$CHCH_2$-PEO_n-OCH_3) [36]. We have previously reported theuse of PEO-silane amphiphiles (PEO-SAs) for the bulk modification of silicones that achieved "on demand" hydrophilicity. Originally developed for RTV silicones, PEO-SAs were comprised of a PEO segment, a hydrophobic oligo(dimethyl siloxane) tether and a triethoxysilane (Si–H) end group that may crosslink with the silicone: $(EtO)_3$-Si-$(CH_2)_3$-$(ODMS)_n$-*block*-PEO_m-OCH_3 [37]. These PEO-SAs produced water-driven surface hydrophilicity that was not observed for the corresponding non-amphiphilic PEO-silane [$(EtO)_3$-Si-$(CH_2)_3$-PEO_n-CH_3]. PEO-silane amphiphiles' unique restructuring capacity was attributed to the flexible oxygen-dimethylsiloxane (ODMS) tether and the improved compatibility within the silicone matrix. Moreover, a PEO segment of $n = 8$ and an ODMS tether of $m = 13$ or 30 showed optimal restructuring [38–40] and resistance to adhesion of proteins and bacteria [41] as well as whole blood [41]. The longer tether ($m = 30$) was associated with somewhat improved retention with continued aqueous exposure (2 weeks). More recently, silane (SiH)-terminated PEO-SAs (HSi-$ODMS_{30}$-*block*-PEO_8-OCH_3) were developed for Sylgard 184, a commonly used addition cure silicone resin. Modified Sylgard (7–14 wt.% PEO-SA) was used to create microfluidic channels that achieved pumpless, capillary-driven flow of blood due to the improved surface wettability [43]. Retention of water-driven surface hydrophilicity was maintained after prolonged ambient storage (6 months). Thus, while other approaches have utilized amphiphilic PEO-PDMS systems [16,19,44,45], these PEO-SA SMAs offer a uniquely practical and robust strategy for imparting surface hydrophilicity to silicones.

Herein, the utility of Sylgard 184 modified with the PEO-SA (HSi-$ODMS_{30}$-*block*-PEO_8-OCH_3) was evaluated for the first time to assess its potential for cell-based and hydrophobic sampling applications. The PEO-SA was incorporated into the silicone at 2%, 9%, and 14% w/w, and the resulting surfaces were examined for compatibility with cell-based assays and studies of small hydrophobic molecules.

2. Materials and methods

2.1. Polymer preparation

The PEO-SA (HSi-$ODMS_{30}$-*block*-PEO_8-OCH_3) (MW = 2778 g/mol) was prepared as previously described in [40]. To prepare the *Pristine PDMS*, Sylgard® 184 (1064291, Dow Corning) base and curing agent were combined in a 10:1 ratio (wt.%) and mixed well. For the preparation of modified silicones formulations, 2, 9, and 14 wt.% (wt.% are expressed in terms of grams of PEO-SA per gram of PDMS) of PEO-SA were added into Sylgard® 184 base and curing agent (10:1 ratio) mixture and placed into a preheated water bath at 60 °C. The mixture was vigorously stirred for 5 min at which point there was a noticeable decrease in the viscosity of the mixture. PDMS + PEO-SA mixtures were poured over the designated mold casting surface and cured in a vacuum oven (90 °C, ~1 mbar) for 1 h. A schematic of this procedure is shown in Fig. 1. Once curing was completed, disks (5 mm diameter) and wells (2 mm internal diameter) used for experiments were prepared using a hole puncher. For removal of uncross-linked oligomers, [1] 20–25 single polymer discs (*Pristine PDMS* or *PDMS + PEO-SA* 2 wt.%, 9 wt.%, or 14 wt.%) were placed in 50 mL centrifuge tubes filled with ethanol (200 proof; Sigma-Aldrich). For sufficient removal of leachable oligomers, specimens were incubated for at least 1 h at room temperature (RT). Disks were then air-dried under laminar flow hood and autoclaved prior to experimental assay. PDMS plasma-treated (*PDMS PLASMA*) was prepared by exposing a *Pristine PDMS* disc to an electric field for 5 min (CORONA PLASMA instrument; BD20-AC).

2.2. FTIR

Attenuated Total Reflectance (ATR) FTIR was performed on the surface and close layers (1.6 μm) from single polymer discs using an FTIR Spectrometer-Spectrum Two (PerkinElmer). The single polymer discs (*Pristine PDMS, Pristine PEO-SA*, *PDMS + PEO-SA* 2 wt.%, 9 wt.%, and 14 wt.%) with ±0.5 mm thickness, were in direct contact with the ATR diamond crystal and scanned 100 times at RT and 90% of gauge pressure; the absorbance measurements were in the range of 500–4000 cm^{-1}. A total of $N = 5$ independent experiments were performed for each *PDMS + PEO-SA* substrate and controls. Results were analyzed using The Unscrambler X v.10.5 (CAMO, Trondheim-Norway) software.

2.3. Absorption of Nile Red

Nile Red (19123, Sigma-Aldrich) was solubilized in PBS 1X (P4417, Sigma-Aldrich) to a concentration of 1 mM. A volume of 3 µL of the solution was added to microwells (~2 mm diameter) made with a hole puncher pressed through a film and placed on a glass slide. Qualitative analysis was done by comparing the stained surface area obtained for each polymer: *Pristine PDMS* and

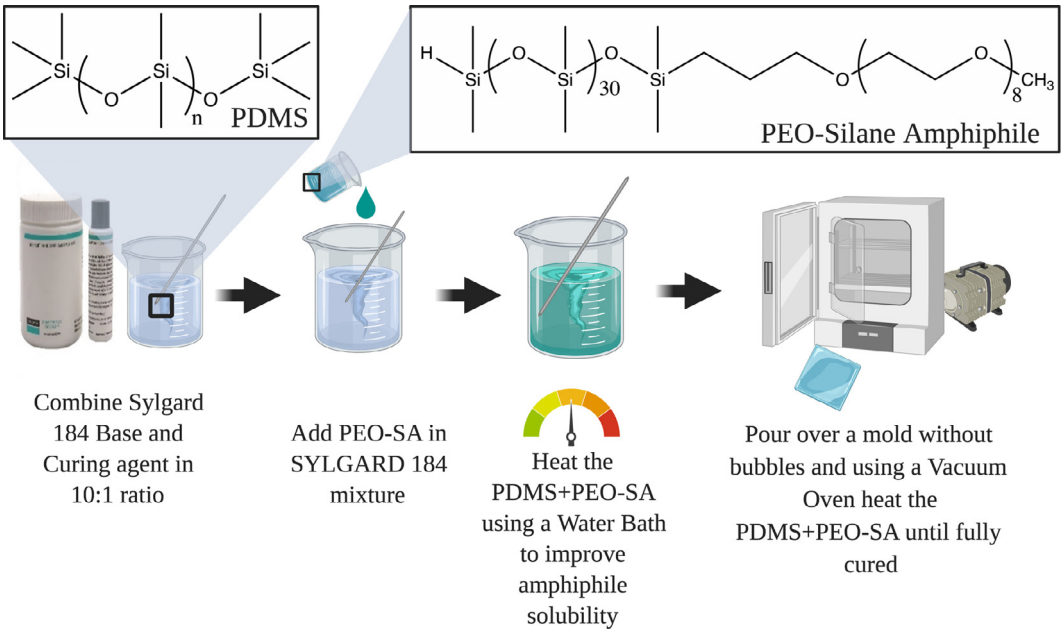

Fig. 1. Schematic of the procedure for the preparation of silicones with PEO-silane amphiphile (*PDMS + PEO-SA*): *Pristine PDMS, PDMS + PEO-SA 2* wt.%, *PDMS + PEO-SA 9* wt.%, *and PDMS + PEO-SA 14* wt.%. Created using Biorender.com.

PDMS + PEO-SAs. Images of each microwell were taken at time intervals of 15 s, 5 min, and 30 min. The surface fluorescence intensity of each polymer sample was analyzed from images collected at 30 min using ImageJ v.1.50i software (NIH, MD- USA).

2.4. Contact angle

A drop volume of 8 μL of sterile DI water was deposited on the surface of each polymer sample. Polystyrene (*PS*) was used as a reference control of hydrophobicity. The contact angle of a liquid water interface was measured over a 5 min period. Next, the polymer samples were stored in a sealed container kept at ambient conditions (25 °C) and aged for up to 3 months (in air) to quantify the stability of the hydrophilic surface properties over time. The contact angle of a water droplet was recorded 5 min after it was deposited, and then this process was repeated at intermittent time points on aged specimens.

2.5. Cell culture

MCF7, MDA-MB-231, MDA-MB-468, and NIH-3T3 cell lines were purchased from ATCC. All cell lines were cultured in a cell culture flask containing Dulbecco's Modified Eagle's Medium (DMEM) (D5796, Sigma-Aldrich) with 10% (v/v) Fetal Bovine Serum Heat Inactivated (F4135, Sigma-Aldrich) and Penicillin 100-unit/ml and Streptomycin 100 μg/ml (P4333, Sigma-Aldrich). Cells were cultured and maintained at 37 °C in 5% CO_2. Adherent cell monolayers were dissociated from the culture surface using a 0.25% (v/v) Trypsin-EDTA solution (T4049, Sigma-Aldrich).

2.6. MCF7 response to tamoxifen citrate

Cell response to estrogen and tamoxifen was examined using the estrogen-sensitive MCF7 cell line. Hydrophobic molecules 17-ß-estradiol (E2758, Sigma-Aldrich) and tamoxifen (S1972, Selleck-Chem) were solubilized in ethanol (459836, Sigma-Aldrich) to a stock concentration of 1 mM. To desensitize MCF-7 to estrogen, cell monolayers in cultures flasks were washed 3 times with PBS 1X (P4417, Sigma-Aldrich) and incubated with phenol-free complete culture media composed of DMEM (D1145, Sigma-Aldrich), supplemented with 10% (v/v) Fetal Bovine Serum Charcoal Stripped (F6765, Sigma-Aldrich) and L-Glutamine with 100-unit Penicillin/ml, and 100 μg Streptomycin/ml (P4333, Corning) at 37 °C in 5% CO_2 for 72 h. Phenol red-free 0.05% (v/v) Trypsin-EDTA solution (59418C, Sigma-Aldrich) was used to dissociate adherent cells from the culture surface. The concentration of viable cells for initial seeding was determined by staining with 0.4% Trypan Blue solution (T8145, Sigma-Aldrich) and manually counted using a hemocytometer. Cells were suspended in culture medium to seed a total of 15,000 cells/well in a 96-well plate. After overnight incubation, the culture medium was removed and replaced with fresh phenol-free culture media supplemented with 1 µM 17-ß-estradiol (E2758, Sigma-Aldrich) and 1-100 µM tamoxifen citrate (S1972, Selleck-Chem). After performing a media change, one polymer disk of 5 mm diameter was placed on the surface of the culture media inside the well. Cells were then incubated for 72 h prior to the examination of cell viability.

2.7. Cell viability

The viability of NIH3T3, MDA-MB-231, MDA-MB-468, and MCF7 cells was examined after a 48 h exposure with *PDMS + PEO-SA* substrates and controls. A total of 30,000 cells suspended in DMEM complete media containing phenol-red were added to each well of a 96 well plate. After overnight incubation, the culture media was replaced with fresh media, and polymer disks were placed on top of the well. Cells were incubated for 48 h at 37 °C in 5% CO_2.

Viability was determined using the XTT assay. XTT powder (X4626, Sigma-Aldrich) was solubilized to 1.0 g/L in PBS 1X. Menadione (02102259, MP Biomedicals), was added to solubilized XTT to a concentration of 5 µM. Then, DMEM complete culture medium supplemented with XTT-menadione (200 μL culture medium/50 μL XTT-menadione) was added to cultures wells and incubated for 2–4 h at 37 °C in 5% CO_2. A volume of 100 μL of culture medium/XTT-menadione was transferred in a new 96-well plate for the absorbance measurements at 465 nm wavelength using a UV/VIS Spectra Multiplate Reader (INFINITE 200 PRO, TECAN).

2.8. Tamoxifen absorption quantification by HPLC

Tamoxifen was diluted to 100 µM in Molecular Grade water (46-000-CI, Corning). Volumes of 200 µL were then placed in 96-well plates with 5 mm polymer disks placed on top. The solution was left for incubation 72 h at 37 °C in 5% CO_2. Tamoxifen absorption was determined using Reverse Phase HPLC with a C-18 column (00G-4041-E0, Phenomenex). The HPLC instrument used was the Agilent 1100 series with a UV-VIS detection system. Clarity software (Version 7.4, DataApex Ltd.) was used for the acquisition and analysis of chromatograms. The separation column used was a Luna 5 µm C18 100A 250 × 460 mm (00G-4041-E0, Phenomenex). The mobile phase consisted of acetonitrile (34998, Sigma-Aldrich) and distilled-deionized water. Trifluoroacetic acid (30203, Sigma-Aldrich) was added to a concentration of 0.1% to both mobile phases. The mobile phase gradient used for separation started with 90:10 (water:acetonitrile) and reached 100:0 after 15 min followed by 2 min for complete elution and 2 min of column equilibration at 90:10. The flow rate was maintained constant at 1.0 mL/min and the temperature was set to 25.0 °C.

2.9. Statistical methods

Statistical analysis was performed using GraphPad Prism Software (version 8, Prism). The statistical test employed was One-Way ANOVA with Tukey Test and CI = 95% for the multiple comparisons. The Student's *T*-test with Dunnet test and CI = 95% was used for single comparisons as appropriate.

3. Results

3.1. PDMS + PEO-SA characterization

3.1.1. Spectroscopy characterization

ATR-FTIR was utilized to assess the surfaces of Sylgard 184 modified with 2%, 9% and 14% w/w of the PEO-SMA (i.e. *PDMS + PEO-SA 2* wt.%, *9* wt.%, and *14* wt.%). Analysis of the *Pristine PEO-SA* was intended to allow for its spectroscopic identification at the surface of the mixed polymers. Nonetheless, the FTIR spectra of *PDMS + PEO-SA* specimens were complex due to overlapping signals attributed to the similarities in functional groups present in both polymers (Fig. 2a). For this reason, a Second Derivative Spectra was generated and smoothed using the Savitsky-Golay algorithm with 7 points to improve the resolution of the analysis (Fig. 2b–e). The identification of the representative functional groups for *Pristine PDMS* and *Pristine PEO-SA* are shown in Table 1.

Table 1
Assignment of IR signals in *Pristine PDMS* and *Pristine PEO-SA*.

IR Bands	Pristine PDMS (cm^{-1})	Pristine PEO-SA(cm^{-1})
Si–CH_3 stretching	2990	2990
Si–CH_3 deformation	1240	1240
Si–O–Si stretching	1120–1010	1120–1010
C–C–O group	–	915–865
Si–CH_3 stretching	840–770	840–770

The siloxane vibrational band (Si–O–Si) is a predominant signal at 1010 cm^{-1} since this functional group is present in both *Pristine PDMS* and the PEO-SA. For the Si–O–Si signal, an increment in the signal was observed at increasing concentrations of PEO-SA (Fig. 2c). Also, the presence of PEO-SA in the modified PDMS spectra was confirmed by monitoring the slight signal produced by the characteristic C–C–O group (915–865 cm^{-1}) only present in compounds modified with PEO. The characteristic signals of the C–C–O group are shown in Fig. 2d and e. An increasing tendency of the signal was observed in the 915 cm^{-1} region (Fig. 2d) where substrates containing PEO-SA showed a higher signal when compared to the Pristine PDMS. When the 865 cm^{-1} signals were compared in Fig. 2e, the previously described correlation was changed and it

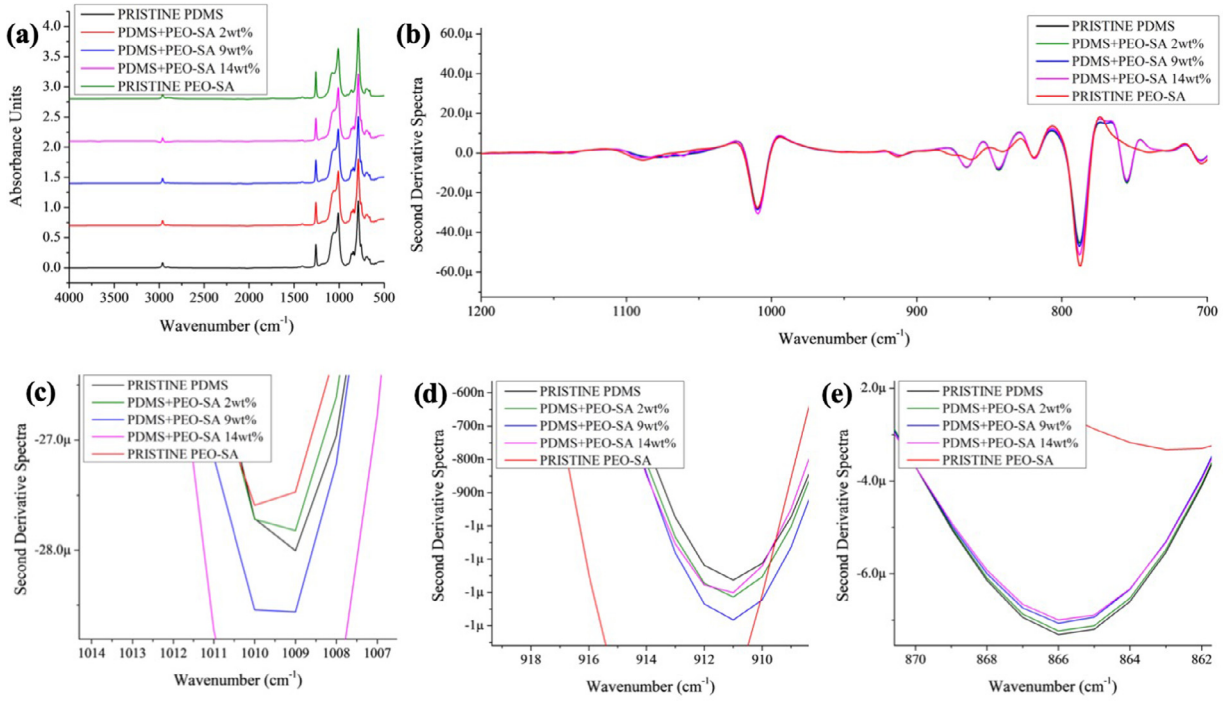

Fig. 2. For *Pristine PDMS*, *Pristine PEO-SA*, and *PDMS + PEO-SA 2* wt.%, 9 wt.%, and 14 wt.%: (**a**) FTIR spectra. (**b**) Second Derivative Spectra (1200–700 cm^{-1} range). (**c**) Si–O–Si Siloxane IR vibrational band. (**d**) C–C–O IR vibrational band. (**e**) Si–CH_3 IR vibrational band.

showed a slight increase inversely proportional to the increase in PEO-SA concentration on the surface of the PDMS. This inverse relation is due to the incorporation of PEO-SA which slightly reduces the Si–CH_3 group in the bulk polymer. In this evaluation, the peaks of PDMS and PEO-SA are at the extremes, and the increase in concentration follows an apparent trend towards the PEO-SA, confirming the presence of PEO-SA in the polymer.

The data was also evaluated by Principal Component Analysis (PCA, **Figure S1**) to visualize relationships between signals and polymer compositions. Qualitative assessment of the PCA confirmed the differences in polymer composition as a result of PEO-SA addition while at the same time provided insight of low variability in the polymer composition from a macroscopic standpoint.

3.1.2. Surface wettability

The ability of the PEO-SA to impart water-driven surface hydrophilicity, including after aging, was examined by contact angle analysis. First, the contact angle of a water droplet deposited on freshly prepared specimens was evaluated over a period of 5 min (Fig. 3a). Initially, the contact angle had an average value of 100°, 26° and 16° for *PDMS + PEO-SA* 2 wt.%, 9 wt.% and 14 wt.%, respectively. The *PDMS PLASMA* control condition had the smallest contact angle which was below 15°. The contact angle measurements remained constant for both, *Pristine PDMS* (~114°) and *PS* (~85°). These results indicate that the addition of PEO-SA (at concentrations of 9 and 14 wt.%) effectively reduced the surface hydrophobicity of Pristine PDMS. The surface contact angle was also recorded on specimens stored at ambient conditions and aged for hours up to 3 months to determine the stability of the surface wettability (Fig. 3b). As expected, *PDMS PLASMA* had hydrophobically recovered and exhibited a contact angle similar to *Pristine PDMS* at 24 h. Conversely, the water contact angles of *PDMS + PEO-SA* 9 wt.% and *PDMS + PEO-SA* 14 wt.% were maintained at ≤25° and ≤19°, respectively, for specimens aged up to 3 months. The retention of these low water contact angle values indicates a stable modification of the surface hydrophilic properties of the Sylgard 184. Due to the enhanced hydrophilicity of PDMS + PEO-SA, it is very likely that its swelling properties were modified. As such, polymer swelling was examined in response to an organic solvent. As expected, it was observed that *Pristine PDMS* and *PDMS + PEO-SA* 2 wt.% showed deformation upon addition of toluene (**Supplemental Video SV1**). On the other hand, *PDMS + PEO-SA* 9 wt.% and 14 wt.% did not show such deformation confirming that PEO-SA changed silicones' swelling properties.

In addition to the examination of surface wettability, other tests were performed to identify potential changes in the mechanical and optical properties of *PDMS + PEO-SA* (**Figure S2**). Specifically, polymer elasticity and optical transparency were examined in response to a constant load and various wavelengths, respectively. PEO-SA incorporation did not lead to noticeable changes in the elasticity as the curves were very similar as compared to *Pristine PDMS* (**Figure S2a**). Similarly, absorbance measurements were not affected across polymers (**Figure S2b**) indicating the retention of the optical properties in *PDMS + PEO-SA*.

3.1.3. Nile Red dye absorption

Nile red fluorescent dye was used to visually confirm the presence of hydrophobic regions at the surface interface with an aqueous solution. Nile Red is a small (MW = 318.37 g/mol) lipophilic fluorophore that fluoresces in the presence of a hydrophobic-rich environment but not in an aqueous solution. This fluorescent behavior of Nile Red allowed us to qualitatively characterize the hydrophobicity of the films. Images depict the fluorescence intensity of absorbed Nile Red after 30 min of contact (Fig. 3f). Images were also sequentially captured at the preceding 15 s and 5 min time points (**Figure S3**). Attributed to its hydrophobicity, the *Pristine PDMS* emitted a greater fluorescence versus *PDMS + PEO-SA* specimens. As the PEO-SA content was increased from 2 to 14%, fluorescence was diminished. Visual inspection of fluorescence images of *PDMS + PEO-SA* 2 wt.% and 9 wt.%, show only small hydrophobic regions at 15 s but that diminished at 5 min, suggesting a greater reorganization of PEO-SA to the aqueous interface with additional time. The fluorescence of Nile Red was quantified using image-based analysis of the fluorescent surface area at 30 min (Fig. 3e). The average measurements of the fluorescence surface area marked by Nile Red, showed a reduction in the surface hydrophobicity of 17.2% for the *PDMS + PEO-SA* 2 wt.%, 71.6% for the *PDMS + PEO-SA* 9 wt.% and 85.4% for the *PDMS + PEO-SA* 14 wt.% as compared to the *Pristine PDMS*. At just 2 wt.%, there is no significant reduction in the Nile Red fluorescent area, yet at 9 wt.% and 14 wt.% the reduction was significant (*P*-value <0.0001). This reduction in fluorescence is in agreement with the increased wettability of the surface observed during contact angle analysis (Fig. 3a). The distribution of Nile Red is not entirely homogeneous. In prior work, atomic force microscopy (AFM) revealed the water-driven restructuring of PEO segments to the aqueous interface of PDMS modified with a PEO-SA, producing a nanocomplex topography [46]. This may contribute to the heterogeneity of the Nile Red diffusion. Nonetheless, the overall uptake is highly reduced by the presence of the PEO-SA, particularly at higher concentrations in the PDMS.

3.2. Polymer cytocompatibility and suitability for drug assays

3.2.1. Cytocompatibility and cell adhesion

MCF7, MDA-MB-231, MDA-MB-468 and NIH-3T3 cell cytotoxicity was evaluated at 48 h post-incubation with polymer discs (Fig. 4a). The average values obtained were normalized to tissue culture plastic (*TCP*, no polymer disc floating) for comparisons against the standard culture control. For MCF7 cells, cell viability values in *Pristine PDMS*, *PDMS + PEO-SA* 2 wt.%, 9 wt.% and 14 wt.% were similar to *TCP*. For MDA-MB-231, MDA-MB-468 and NIH-3T3 cells, *Pristine PDMS* and *PDMS + PEO-SA* 2 wt.% had similar viability versus the TCP control. For, *PDMS + PEO-SA* 9 wt.% and 14 wt.%, a small (<10%) but significant decrease in viability was detected. Despite this, the *PDMS + PEO* 9 wt.% and 14% were considered non-cytotoxic towards these cell types based on >85% viability. The morphology of these cells was also observed to be similar across substrates (**Figure S4**). Since silicones modified with PEO-SA have been shown to resist protein adsorption [39-42], the adhesion of MCF7 cells to the *PDMS + PEO-SA* substrates was also examined to determine to what extent this protein resistance will affect cell adhesion (**Figure S5**). Total cell counts indicated that the amount of adhered cells was reduced by ~93% on *PDMS + PEO-SA* 9 wt.% and 14 wt.% versus *Pristine PDMS* indicating that the polymer will require priming of the surface in order to be used as cell culture substrate.

3.2.2. Tamoxifen absorption and IC_{50}

The concentration of tamoxifen, a small hydrophobic drug, was quantified in an aqueous environment to determine the amount being absorbed following incubation with specimen disks (Fig. 4b). Results showed a positive correlation of tamoxifen concentration in aqueous solution and PEO-SA concentration. A significant decrease in tamoxifen absorption of ~18% was observed for *PDMS + PEO-SA* 14 wt.% as compared to *Pristine PDMS* (Fig. 4c) indicating that PEO-SA reduces tamoxifen sequestration from an aqueous environment. To determine whether the sequestration of tamoxifen into the specimen discs was perceived at the cell level, the IC_{50} of tamoxifen was estimated in cells using the tamoxifen-sensitive MCF7 cell line. The individual IC curves are shown in **Figure S6** and summarized in Fig. 4d. TCP was used as a reference control

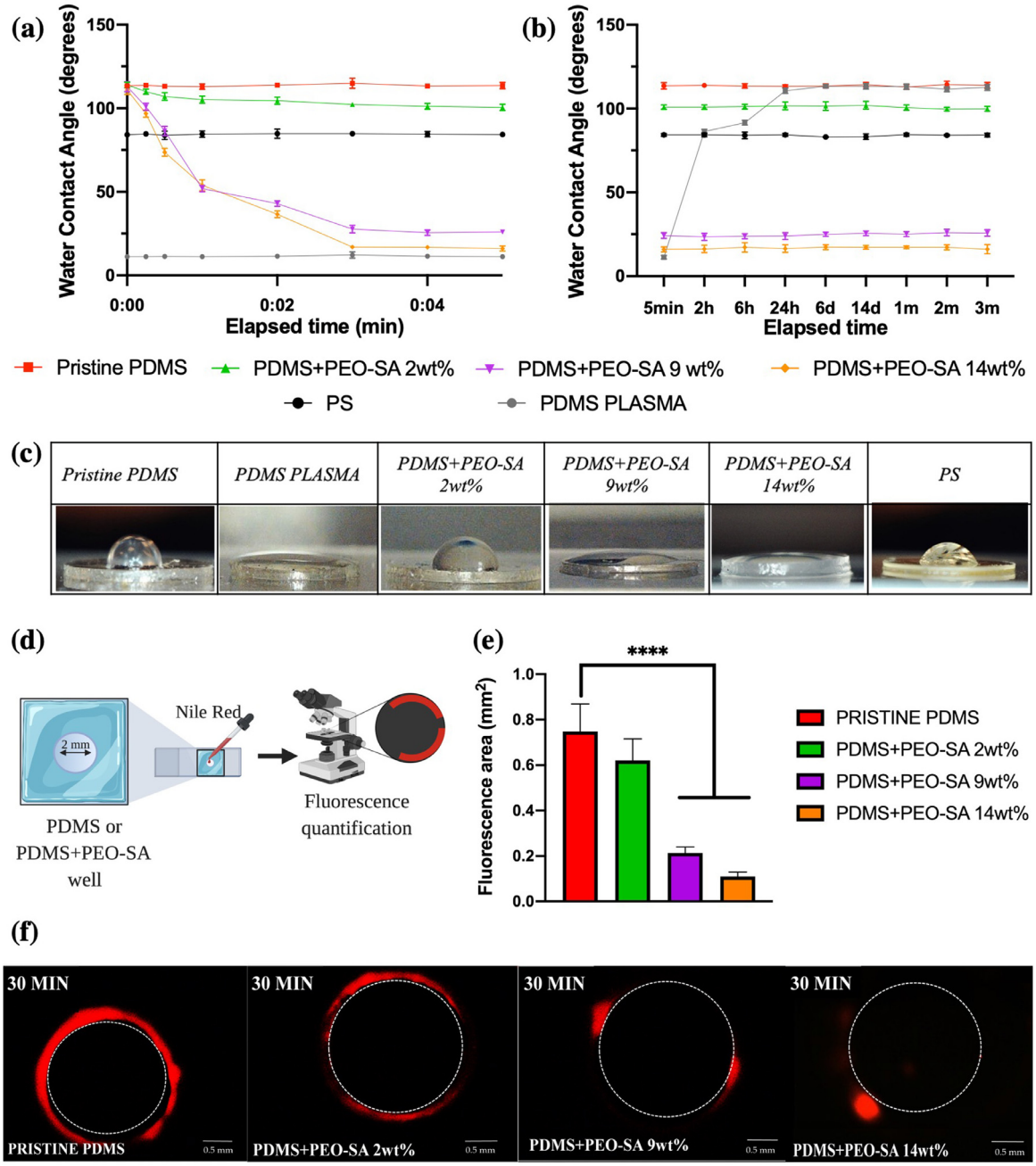

Fig. 3. *PDMS + PEO-SA wettability and absorption properties*. A volume 8 μL water droplet on *Pristine PDMS, PDMS PLASMA, PDMS + PEO-SA 2* wt.%, *9* wt.%, and *14* wt.%, and *PS*. **(a)** For freshly prepared specimens, water contact angle measurements taken in intervals over 5 min. (**b**) Water contact angle measurements were recorded at 5 min and then on specimens aged in air for hours (h), days (d), and up to 3 months (m). Each point represents the average of three water droplet measurements on three different specimens at the same point. (**c**) Photos of water droplets deposited on specimen surfaces. **(d)** Schematic of the Nile Red absorption assay. Wells (inner diameter of 2 mm) were prepared in a film with a hole puncher and placed over a glass slide. A 3 µL drop of Nile Red diluted in water was added to each well. Images were taken using a fluorescence microscope and fluorescent area was quantified using NIH Image-J software. (**e**) The average fluorescence area of Nile Red absorbed into hydrophobic regions of polymer wells at 30 min post-incubation. Data shown represent the average ± SD of $N = 3$. Statistical analysis was done using single-factor analysis of variance (ANOVA), $^{****}P < 0.0001$. **(f)** Representative fluorescence images of Nile Red absorbed at 30 min into polymer wells. The boundaries of each well that contain the aqueous Nile red solution are marked with a white dashed-line of a 2 mm diameter. The scale bar in the image corresponds to 0.5 mm.

for the growth-inhibitory activity of tamoxifen. The IC_{50} obtained for TCP had an average value of 19.7 µM +/− 2.2 µM which is within the range of the average value reported on the Genomics of Drug Sensitivity in Cancer database [47]. As expected, the IC_{50} obtained for *Pristine PDMS* was above TCP with an average value of 34.9 µM +/− 9.0 µM which is 77.2% higher than the value obtained in TCP (Fig. 4d). This increase in IC_{50} value is in accordance with previous reports showing an absolute absorption of tamoxifen into *Pristine PDMS* when compared to *TCP* [48]. The IC_{50} of tamoxifen for *PDMS + PEO-SA* 9 wt.% was 21.0 µM +/− 8.5 µM which represents a 40% reduction of the value obtained for *Pristine PDMS*, and equivalent to the value obtained in TCP. Although a full recovery of the concentration of tamoxifen in aqueous solution was expected, probably the presence of some hydrophobic pockets in the surface of *PDMS + PEO-SA 9* wt.% and *14* wt.% contributed to some of the variability observed in the reported IC_{50} as supported by previous absorption results shown in Fig. 4c and Fig. 3d. Yet, the reduced absorption of tamoxifen into *PDMS + PEO-SA 9* wt.% and *14* wt.% was sufficient to fully recover the growth inhibitory activity and toxicity of tamoxifen in MCF7 cells on TCP.

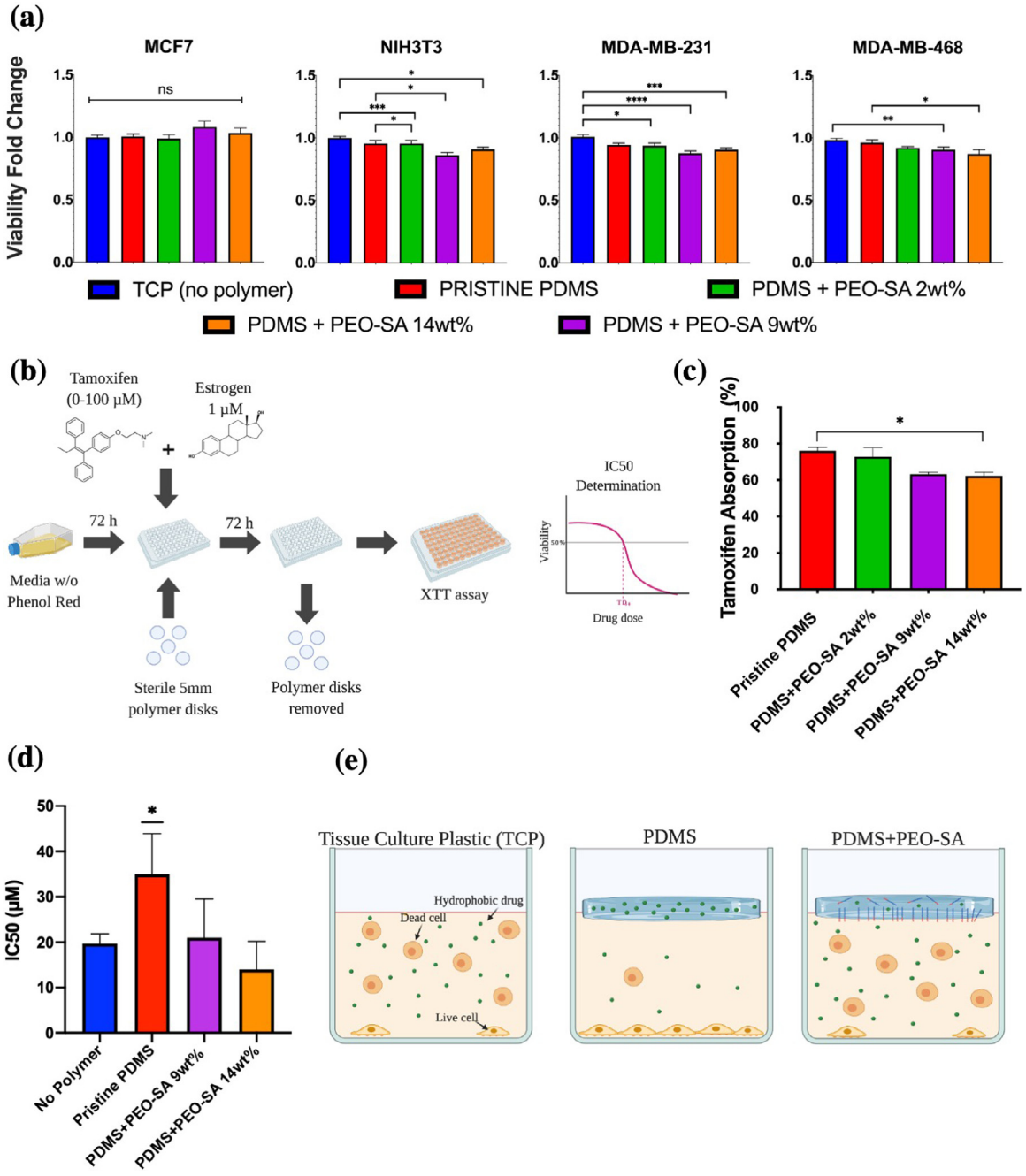

Fig. 4. *Cell viability and Tamoxifen drug assay in MCF-7 cells.* **(a)** Viability experiments were performed 48 h post-treatment. Single specimen discs were added to each well of a 96-well plate. The data was normalized with respect to the TCP. Data represents an average of 3–5 independent experiments with $N = 4–6 \pm 1$ SEM. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns = $P > 0.05$, *= $P < 0.05$, **= $P < 0.01$, ***=$P < 0.005$, ****=$P < 0.0001$. (**b**) Schematic of the IC_{50} assay for tamoxifen toxicity in the MCF7 cell line. (**c**) Tamoxifen absorption into polymer disks was determined using Reverse Phase HPLC. Results show the average and SEM of $N = 3$ replicates per polymer. (**d**) Summary of results from IC_{50} assay. Data shown represent the average and SEM after $N = 4$ for *TCP, PDMS + PEO-SA 9* wt.% and *14* wt.% and $N = 3$ for Pristine PDMS. Results were compared using Student's *T*-test with CI = 95%. (**e**). Schematic explanation of the results obtained in our IC_{50} determination experiment. Created using Biorender.com.

4. Discussion

4.1. PEO-SA stably reduces surface hydrophobicity and small molecule absorption

A limited amount of biochemical modifications have been shown to demonstrate long-term wettability enhancement of silicones. Previous modifications to PDMS for cell culture applications including treatment with ionizing radiation [49] and Parylene-C surface coating [48]. More common surface modifications such as plasma etching or ionic detergent surface coating, are easy and fast to implement during fabrication but the surface hydrophobicity is recovered within hours or days [50]. *PDMS + PEO-SA* showed a surface wettability that was stable for at least 3 months which is superior as compared to the standard oxygen plasma etching method and other reported modifications including most of the surface grafting, silanization and layer-by-layer deposition methods [15]. Thus, these approaches are insufficient to prevent the absorption of small hydrophobic molecules from aqueous solutions that will negatively impact analytical assays. In this work, the use of the

amphiphilic PEO-SA is supported as an alternative to readily stably reduce the surface hydrophobicity of PDMS substrates in aqueous environments. Notably, the PEO-SA can be readily blended into the PDMS and substrates prepared with no further modification to the fabrication process. *PDMS + PEO-SA 9* wt.% and *14* wt.% exhibited dramatically enhanced surface hydrophilicity that was retained for 3 months aging in air. Additionally, hydrophilicity was confirmed by the substantial reduction in the uptake of Nile Red from aqueous solutions. In these experiments, a few small regions of Nile Red absorption were observed. As noted, water-driven surface-reorganization of PEO segments of the PEO-SA generates a complex nanotopography of PEO-rich domains [46]. This may contribute to the somewhat heterogeneous Nile Red absorption. This behavior may be potentially mitigated with enhanced mixing (e.g. high speed or increased temperatures) of the PDMS and PEO-SA. Despite this, PDMS surface hydrophilicity was greatly enhanced with the addition of the PEO-SA. This enhanced hydrophilicity was further confirmed by exposure to a hydrophobic organic solvent (toluene) in which *PDMS + PEO-SA* compositions showed greater swelling resistance with increasing PEO-SA concentration (**Supplemental Video SV1**).

4.2. PEO-SA suitable for cell-based assays

The absorption of small hydrophobic molecules into silicones can induce platform-related artifacts [1,48], thereby limiting its application in drug potency assays and sampling applications. In our previous study, we showed that PDMS had the capacity to absorb small hydrophobic molecules from the cell culture media that negatively impacted signaling response to estrogen hormone [1]. Similarly, other studies have shown that PDMS can absorb small hydrophobic molecules even after multiple rounds of volume replenishment [51], further highlighting its capacity to deplete the media from small hydrophobic molecules. Our studies here support the use of *PDMS + PEO-SA* to significantly reduce the absorption of small hydrophobic molecules while retaining the optical and cell-culture cytocompatibility properties of silicones. For both cancer cells and fibroblasts, cell viability was maintained at levels above 85% . The examination of optical transparency and elasticity showed similar values for Pristine PDMS and all other PEO-SA modifications, indicating that the optical and mechanical properties were not significantly affected (**Figure S1**). PEO-SA reduced the absorption of tamoxifen in PDMS at concentrations of 9 and 14 wt.%. Moreover, the drug toxicity assessment using tamoxifen showed a full recovery of the cellular toxicity in *PDMS + PEO-SA* samples compared to TCP and PDMS. Thus overall, the absorption of small hydrophobic molecules was significantly improved and the data shown support the compatibility of PDMS + PEO-SA for cell studies and drug assays.

4.3. New directions

PDMS is one of the polymers employed in flexible microfluidic interfaces used for biosensing and mechanical sensing applications in both implantable and wearable devices [52,53]. Yet, its surface hydrophobicity and porous surface will likely impair accurate and timely measurements, particularly for hydrophobic analytes, by limiting the amount of analyte that reaches the sensing unit [54]. This is a limitation in the field of microfluidic sampling particularly for hydrophobic factors such as steroid hormones, lipids and metabolites that are intended to be detected and quantified by non-invasive sampling of fluids [54]. As demonstrated herein, PEO-SAs are effective SMAs for PDMS and can readily be incorporated with simple bulk modification rather than with a complex surface modification strategy. As demonstrated in our prior work, unlike traditional PEO, the amphiphilic nature of the PEO-SAs uniquely leads its rapid and extensive water-driven migration to the aqueous interface to render the surface highly hydrophilic. This biochemical property can be used for enhanced sampling in wearable devices. For example, PDMS + PEO-SA could be used as a polymer interface for guiding hydrophobic molecules present in aqueous solutions towards the sensing unit in microfluidic devices. One potential aqueous fluid of interest is sweat because it contains hydrophobic molecules, such as cortisol and certain proteins, that are present in low concentrations and will be challenging to detect from a PDMS sampling interface. Also, certain drugs, or products of drug metabolism, have also been found in sweat, adding potential applications of *PDMS + PEO-SA* polymer for sampling and monitoring drugs in sweat [55,56]. As such, PDMS + PEO-SA has the potential to expand the limited breath of biomolecules that can be sampled using conventional PDMS surface interfaces.

Other polymer properties *of PDMS + PEO-SA* that fell outside this study's focus remain to be examined in depth to determine the extent of potential applications in the biomedical field. For example, the mechanical and electrical properties are relevant for digital biosensor development using PDMS [57–59], and the impact of PEO-SA in such properties of pristine PDMS remains to be determined. Also, other studies have characterized PDMS properties such as capacitance and resistivity to develop temperature and pressure sensors [59–61]. These properties of PDMS may be affected by the PEO-SA incorporation due to potential changes in the molecular composition and molecular mobility of the bulk polymer. Along with a deeper understanding of polymer properties, further works could also elucidate other applications that could benefit from the use of PDMS with PEO-SA.

5. Conclusion

In conclusion, the addition of PEO-SA to PDMS stably enhanced surface wettability for up to 3 months. We were also able to demonstrate reduced drug absorption and biocompatibility of PDMS + PEO-SA for cell and drug-based applications. Its enhanced and stable surface wettability will expand the breadth of technical applications for PDMS + PEO-SA for studies of hydrophobic molecules in aqueous solutions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2020.11.041.

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