TARGETING PDMS HYDROPHOBICITY FOR STUDY OF SMALL HYDROPHOBIC MOLECULE-DRIVEN CELL RESPONSES IN VITRO

by
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ABSTRACT

Poly(-dimethyl siloxane) (PDMS) has been the main polymer employed in micro and nano scale devices. Its chemical and physical properties are ideal for molding and soft patterning of microfluidic devices. Despite the great advances achieved with PDMS-based microfluidic devices, its hydrophobic properties have been a main limitation for its routine implementation in standard cell biology studies and drug assays. Chemical and physical modifications of PDMS have been shown to reduce the hydrophobicity of PDMS but display incompatibility with cell culture applications due to recovery of hydrophobicity.

Therefore, to overcome some of the challenges associated with PDMS hydrophobicity, we evaluated a novel method for reducing the hydrophobicity of the bulk PDMS for cell culture and cell-based assays. In our method, a biocompatible oligomer, polyethylene oxide silane amphiphile (PEO-SA) was incorporated into the bulk PDMS to different concentrations: 2wt%, 9wt% and 14wt%. PEO-SA is one of the most used polymer additives for enhancing the hydrophilicity of polymers and decrease adsorption in hydrophobic stable substrates such as silicones. The incorporation of PEO-SA at different concentrations into the PDMS was analyzed by spectroscopy FTIR, and measurements of the surface contact angle on each substrate. Optical transparency, drug absorption and impact on viability assays, and biocompatibility were done to select those PDMS modifications that best reduced hydrophobic absorption without negatively impacting cell behavior.

Results obtained showed that PEO-SA was incorporated into the PDMS reducing it hydrophobicity and surface properties over 3 months. The optical transparency of the PDMS was not altered when modified with PEO-SA. Small molecule absorption was qualitatively and indirectly evaluated by fluorescent microscopy and cell viability analysis, showing that there was a reduction in absorption of hydrophobic molecule proportional to the concentration of PEO-SA. Biocompatibility was analyzed by viability and proliferation assays, which corroborate a no toxicity of the modified PDMS, though a reduction in the cell proliferation was modulated by addition of PEO-SA above 9wt%.

PDMS+PEO-SA 2wt% is a potential option for replacing the pristine PDMS for cellular assays in which small hydrophobic molecules are involved.

RESUMEN

El poli (-dimetil siloxano) (PDMS) ha sido el principal polímero empleado en dispositivos en micro y nano escala. Sus propiedades químicas y físicas son ideales para el modelado de dispositivos microfluídicos. A pesar de los grandes avances logrados con estos dispositivos basados en PDMS, sus propiedades hidrofóbicas han sido una limitación principal para su implementación cotidiana en estudios de biología celular estándar y ensayos de fármacos. Se ha demostrado que las modificaciones químicas y físicas del PDMS reducen su hidrofobicidad, pero muestran incompatibilidad con las aplicaciones de cultivo celular debido a la recuperación de la hidrofobicidad.

Por lo tanto, para superar algunos de los desafíos asociados con la hidrofobicidad del PDMS, se evaluó un nuevo método para reducir dicha propiedad, y así, extender sus aplicaciones en ensayos celulares. En nuestro método, un oligómero biocompatible, óxido de polietileno silano anfifílico (PEO-SA por sus siglas en inglés) se incorporó en el PDMS. PEO-SA es uno de los aditivos poliméricos más utilizados para mejorar la hidrofilicidad de los polímeros y disminuir la adsorción en sustratos estables hidrofóbicos como las siliconas. La incorporación de PEO-SA a diferentes concentraciones en el PDMS se analizó mediante espectroscopía FTIR y mediciones del ángulo de contacto superficial en cada sustrato. La transparencia óptica, la absorción del fármaco y el impacto sobre los ensayos de viabilidad, y la biocompatibilidad se realizaron para seleccionar aquellas modificaciones de PDMS que mejoran la reducción de la absorción hidrofóbica sin afectar negativamente al comportamiento celular.

Los resultados obtenidos mostraron que PEO-SA se incorporó al PDMS reduciendo su hidrofobicidad y propiedades superficiales durante 3 meses. La transparencia óptica del PDMS no se alteró cuando se modificó con PEO-SA. La absorción de moléculas pequeñas se evaluó cualitativa e indirectamente mediante microscopía de fluorescencia y análisis de viabilidad celular, demostrando que hubo una reducción en la absorción de dichas moléculas, proporcional a la concentración de PEO-SA. La biocompatibilidad se analizó mediante ensayos de viabilidad y proliferación, los cuales corroboran una no toxicidad del PDMS modificado, aunque la adición de PEO-SA por encima del 9% en peso moduló una reducción en la proliferación celular. PDMS + PEO-SA 2% en peso es una opción potencial para reemplazar el PDMS prístino para ensayos celulares en los que están implicadas pequeñas moléculas hidrófobas.

To God for his many undeserved blessings, for helping me in every step I take.

To my wife and girl for their love and constant support, and to my extended family for their prayers.

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GLOSSARY OF TERMS

%wt	*Percentage by weight*
2D	*Two-dimensional*
3D	*Three-dimensional*
APTES	*(3-Aminopropyl) triethoxysilane*
ATR	*Attenuated Total Reflectance*
CVD	*Chemical Vapor Deposition*
Da	*Dalton, units of molecular weight (g/mol)*
DOX	*Doxorubicin*
DOX.HCL	*Doxorubicin Hydrochloride*
ER	*Estrogen Receptor*
FTIR	*Fourier Transform infrared spectroscopy*
HER2	*Human Epidermal Growth Factor Receptor 2*
LBL	*Layer-by-layer*
MTES	*Methyltriethoxysilane*
ODMS	*Oligodimethylsiloxane*
PCA	*Principal Component Analysis*
PDMS	*Poly (-dimethyl siloxane)*
PDMS+PEO-SA	*Blend bulk polymer*
PEO	*Oxide Polyethylene*
PEO-SA	*Oxide Polyethylene-Silane Amphiphile*
PR	*Progesterone Receptor*
PS	*Polystyrene*
PVA	*Polyvinyl alcohol*
SMAs	*Surface-modifying additives*
TEOS	*Triethoxysilane*
UV	*Ultraviolet Radiation*
UV/O	*Ultraviolet and Ozone treatment*
UV/VIS	*Ultraviolet-visible spectral region*
WCA	*Water Contact Angle*

CHAPTER 1 – INTRODUCTION

1.1 ***MOTIVATION***

The microscale technologies have changed the way modern biology is realized and are making substantial contributions to biomedical research. The use of microscale devices in molecular assays has reduced the amount of sample volumes required and cost of reagents providing gains in scalability and improved assay sensitivity[1]. The materials used to fabricate microfluidics device early were from silicon and glass, but impediments were found in cell biology and medical research, as opacity, fragility and incompatibility with microscopy methods. In the 80s elastomeric micro-molding techniques were developed and applied to microfluidics in cell biology assays overcoming main limitations in biocompatibility and optical imaging. Poly(-dimethyl siloxane) (PDMS) has been the main polymer employed in microscale devices. Its chemical an physical properties are ideal for molding and soft patterning of microfluidic devices[2]. Despite the great advances achieved with PDMS-based microfluidic devices, its hydrophobic properties have been a main limitation for its routine implementation in standard cell biology studies and drug assays for pharmaceutical and cell manufacture applications. Absorption of small hydrophobic molecules (<1kDa) into bulk PDMS has been shown to uncontrollably change the concentration of proteins and compounds which introduces experimental variability across replicates and may impact cell behavior[3]. Chemical and physical modifications of PDMS have been shown to reduce the hydrophobicity of PDMS but hydrophobicity is recovered which may introduce artifacts in cell-based studies and sensor platforms [4,5].

To overcome some of the challenges associated with PDMS hydrophobicity, we evaluated a novel method for reducing the hydrophobicity of the bulk PDMS for cell culture and cell-based assays. In our method, a biocompatible oligomer, polyethylene oxide silane amphiphile (PEO-SA)[6–8] was incorporated into the cured of PDMS. Given the compatibility of the chemistry in both silane compounds we expect to improve the wettability of the bulk PDMS without negatively impacting the biocompatibility and optical transparency (comparable to tissue culture plastic and glass substrates) of the material[7]. The studies here describe the

objectives, literature review, methodology and results obtained in the fabrication and characterization of the modified PDMS polymers (PEO-SA *added*) to validate its potential for cell culture applications.

1.2 ***HYPOTHESIS***

Incorporation of PEO-SA in the bulk PDMS will permanently decrease the absorption of small hydrophobic molecules while retaining its biocompatible properties for cell-based assays.

1.3 ***OBJECTIVES***

The main goal of this work is:

- ♣ To develop a biocompatible and long-lasting method to reduce the hydrophobicity of the PDMS for the study of the small hydrophobic molecule-driven cell responses *in vitro*.

The specific objectives of this work are:

- ♣ To confirm PEO-SA incorporation into PDMS substrates
- ♣ To confirm optical transparency and reduction of hydrophobic absorption of small molecules into the PDMS-PEO-SA substrates
- ♣ To determine biocompatibility of the PDMS substrates
- ♣ To confirm that cell response to hydrophobic molecules is improved in PDMS-PEO-SA substrates

1.4 ***SUMMARY OF FOLLOWING CHAPTERS***

In the next chapters will be developed the Literature Review that supports this work (CHAPTER 2), the Methodology used for the fulfillment of the objectives (CHAPTER 3), the Results obtained experimentally (CHAPTER 4), the Conclusion of the results obtained in the search to achieve the objectives set (CHAPTER 5), the REFERENCES cited for the information given and the APPENDIX.

CHAPTER 2 – LITERATURE REVIEW

PDMS is a crosslinked silicone polymer composed of hydrophobic dimethylsiloxane oligomers (Figure 1). It has many attractive physical, chemical and mechanical properties[3] for micro and nano scale fabrication and molecular assay prototyping including easy fabrication, gas and vapor permeability[1], non-toxicity[9], good elasticity[10], high chemical resistance, thermal and oxidative stability, low modulus[6], irreversible bond to different materials, low-cost[11] and optical transparency[9,12]. Currently, PDMS plays a major role in different applications, being preferred by engineers for fabrication of micro and nano fluidic devices[10] employed in molecular assays and cell culture applications ranging from surface micropatterning to the casting of 2D and 3D geometries[3].

a) Curing agent Sylgard 184 (PDMS) b) Base Sylgard 184 (PDMS)

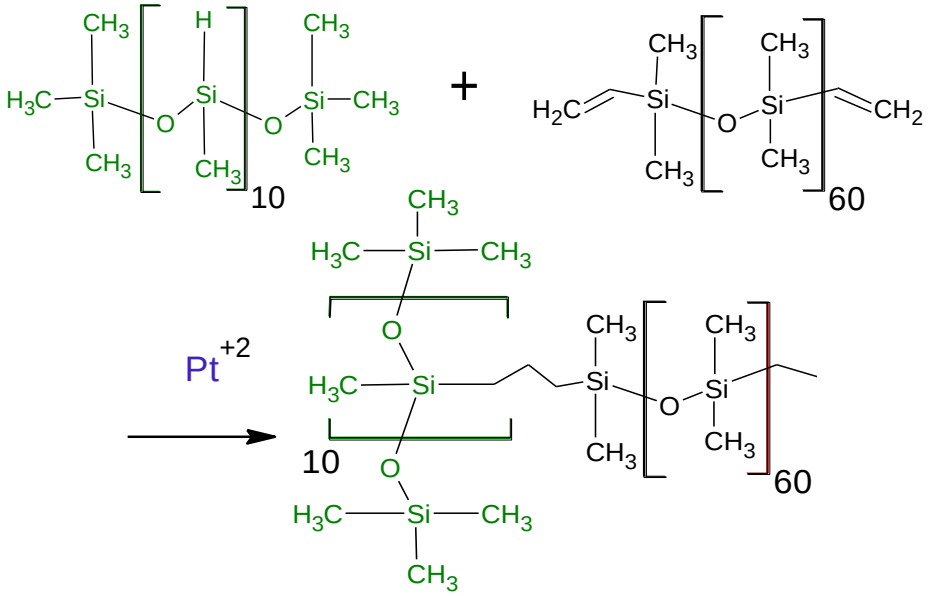

Figure 1. ***Chemistry scheme that leads the cross-linked of PDMS***. *Down Corning Sylgard Elastomer 184 kit contain a base and curing agent with siloxane oligomers a) Curing agent: cross-linking siloxane oligomers and platinum-based catalyst that cure the elastomer. b) Base: cross-linking siloxane oligomers with vinyl groups(*[5]*).*

Despite of these advantages, the PDMS-based microdevices have been shown to leach uncrosslinked[2,3] oligomers and display high adsorption of molecules such as plasma protein (fibrinogen)[6] and marine organisms (coating ships)[13], and the absorption of several others small hydrophobic molecules (<1kDa) sequestered from the culture medium such as steroid hormones[3] in the absence of estrogen and drugs absorption[10,14]. The absorption of uncontrolled amounts of small hydrophobic molecules can shift observed drug potency in dose response assays and influence cell behavior in cellular studies[9,10]. In this regard, several methods have been evaluated to reduce the surface hydrophobicity of PDMS, including physical, chemical and physical-chemical[4] modification.

The physical methods are made by molding micro or nano structures on the PDMS surface to set-up its roughness, wettability by Wenzel and Cassie-Baxter states (for homogeneous and heterogeneous regimes on a rough surface, respectively), and chemical properties shifting the ratio and geometric model of microstructure PDMS[15]. Physical modifications alter patterned geometry which is less desirable in culture applications.

Chemical modifications of PDMS have been more successful in targeting PDMS hydrophobicity but with certain limitations for cell culture applications. The chemical modification has as main methods, plasma grafting, chemical coating, surface-modifying additives (SMAs)[13] introduction of functional groups, UV-generated ozone[4] and oxygen plasma. These last methods are an effective approach to reduce the hydrophobicity, but the hydrophilic property of the treated PDMS is not permanent and will return eventually to the pristine state[16]. Hydrophobic recovery is caused by the surface migration of non-cured PDMS oligomers[17] .

The grafted-modification onto PDMS with others polymers without affecting its biocompatibility or optical properties have been difficult with reversible results in the hydrophobicity[7]. The addition of hydrophilic groups onto PDMS to modify its surface tension such as -OH, COOH, -NH2 and -CO- have been reported previously and show a reduction in the hydrophobicity of the PDMS but the effect is temporary as it only last between 2-24hrs[4]. Main methods used to modify the PDMS are shown in table 1.

Table 1. ***Review PDMS modification methods.***

PDMS MODIFICATION		***DESCRIPTION***	***COMMENTS***	***REF.***
Gas-phase processing	*Plasma treatment*	An ionized gas, such as oxygen, nitrogen or hydrogen, modify the PDMS surface by dissociation and bounding of atoms and molecules with abundant radical species. This modification is considered a suitable skin mimic for the study of permeability for the pharmaceutical industry.	Hydrophilicity was improved (WCA 112º to 60º), influencing the permeability of different compounds. Despite thus, the hydrophobic feature was recovery in a few days.	18,19
	Plasma grafting	Plasma treatment is initially used to break chemical bonds on the PDMS surface. Flows of argon and oxygen are regulated at higher plasma energy levels, for acrylic acid grafting with an excess of argon flow.	Hydrophilic modification on PDMS surface is constant for several days but optical and flexibility properties are deteriorated.	20,21
	UV/Ozone Treatment	Microfluidics devices fabricated with PDMS was treated with UV/O by interactions of UV-light and oxygen from the air atmosphere to produce ozone (185nm UV). The organic moieties reaction with the ozone (254nm UV) to eliminate hydrocarbons on the bulk polymer. The treatment was performed keeping under control the temperature ~100°C to avoid the cracking.	Polymer modification have a depth ~10um and are liable to contraction. Over 100 °C the accumulative stress produced the cracking of the polymer.	22,23
	CVD	The steps for this treatment is sublimation, pyrolysis (690°C) and deposition. The Parylene-C monomers way-out the pyrolysis process and impact the surface of the PDMS when reverse sublimation is carried out, some of the monomers react with free radicals on the surface or in the bulk of the PDMS and are then desorbed outside the process.	Additional oxygen plasma treatment is required to reduce the adsorption of molecules and the hydrophilicity was achieved to ~103° to ~83°.	11,24
	Coating	The PDMS was coated with hydrophilic polymers (PVA and PEG). It is dissolved in water for 40min and the temperature is increased to ~100°C, a reduction on the temperature is applied to ~65°C and left to stir overnight. The solution of hydrophilic polymers was casted onto The PDMS previously treated with plasma and blown dry with nitrogen and heated to ~110°C x15min. The oxygen plasma of PDMS create C-OH, Si-OH and COOH, this groups avoid the hydrogen bonding between the hydrophilic polymers and PDMS.	The long exposition to different plasma treatment of the PDMS cause a reduction in the hydrophobicity, but, also, a decrease in its thermal integrity, leading the cracking of the surface or an unstable bonding reaction with PVA.	25,26

Wet Chemical Methods	*LBL deposition*	The LBL technique is an alternating adsorption of different polycations and polyanions that are consumed on PDMS surface to produce polyelectrolyte layers by ultrasonication.	Long-lasting hydrophobicity reduction (> 2 weeks). However, the functionality of this technique depends of the concentration, temperature, pH, solvent, etc.	[27,28]
	Sol-gel coating	Polymerization method based in a transition phase on a liquid state ("sol") to solid-like state ("gel"). The chemical surface of the PDMS is stable thanks to high density and homogeneous distribution of the "gel". TEOS and MTES are used as precursors, liable to swell and dissolve the PDMS, so it is recommended the oligomerization of the precursors.	The molar ratio between the TEOS/MTES are important to keep good biocompatibility and optical properties without a "significant" modification on the geometry of PDMS.	[29,30]
	Silanization	PDMS contain on the surface hydroxyl groups, which react with APTES to form Si-O-Si bonds. The oxidation of PDMS is performed by immersing in different solvents, as NaOH, HCl or H_2O/H_2O_2 mixture.	The PDMS surface was enhanced (to ~126° to ~77°). Long-term cellular adhesion is related with the inherent PDMS hydrophobicity.	[31,32]
	Dynamic Surface Modification	Surfactants and ionic liquids are used to modify the PDMS surface. These modifiers are added prior the curing PDMS by blending. PEO-based is the Surface-modifying additive (SMA) most used to achieve protein resistance.	The hydrophobicity was reduced (~24°) The silane tether is important to enhance the protein resistance and hydrophilicity on the PDMS.	[6,7,13]
	Deliberate protein adsorption	Hydrophobins, amphiphilic small proteins (<20 KDa), are used to change the wettability of PDMS by coating, allowing the binding of other proteins. PDMS is immersed in Hydrophobins solution at several intervals times.	The PDMS wettability was changed from superhydrophobic to hydrophilic (~123º to ~50º stable for 20 days). Limited for the immobilization of biomolecules.	[33–35]
Combinations	*UV/plasma and Silanization/ graft polymerization/ LBL assembly*	Combination of these methods are carried out with the purpose of reducing the adhesion of proteins on the surface of PMDS microchannels through the reduction of their hydrophobicity.	Effective in reducing the wettability of the PDMS, recording long-lasting ~ 5 months. Despite these great advantages, biocompatibility and complexity of the methods restrict their uses.	[17]

As mentioned before, absorption of small hydrophobic molecules into PDMS is a main limitation for integration of PDMS-based microscale culture platforms in drug screening applications. [9,10,14] The main goal of this project is to modify the bulk PDMS polymer via

incorporation of Poly (-ethylene oxide) Silane Amphiphilic to permanently reduce hydrophobic absorption of PDMS for cellular studies using drug assays.

Poly (-ethylene oxide) (PEO) is, currently, one of the most used polymer additives for enhancing the hydrophilicity of polymers and decrease adsorption in hydrophobic stable substrates[6]. PEO is a biocompatible[36] and hydrophilic polymer commonly incorporated into silicone materials to reduce protein adsorption and hydrophobicity. The surface of the silicones is converted to reactive silanol groups (Si-OH) by different methods such as oxygen/air plasma[37], Ultraviolet radiation[38], UV/Ozone radiation[39], etc. Subsequently, the PEO is grafted onto silicone surface by silanization reaction of PEO-silane with appropriated ending groups in its composition (*i.e.* alkoxysilanes)[40]. PEO is widely used for its properties such as steric repulsion mechanism, blockage of adsorption and a repulsive hydration[6,13].

The length of the siloxane tether is an important determinant in the modification of bulk and surface properties of the polymer. Murthy et, al. (2007) reported the optimization in the incorporation of PEO into silicones via grafting in siloxane tethers and studied the importance of the polymer length on the surface properties. His studies showed that increasing the length of the siloxane tether (n=13) produced a reduction of the hydrophobicity in aqueous environment enabled through mobilization PEO to the surface (Figure 2) of the hydrophobic substrate[8].

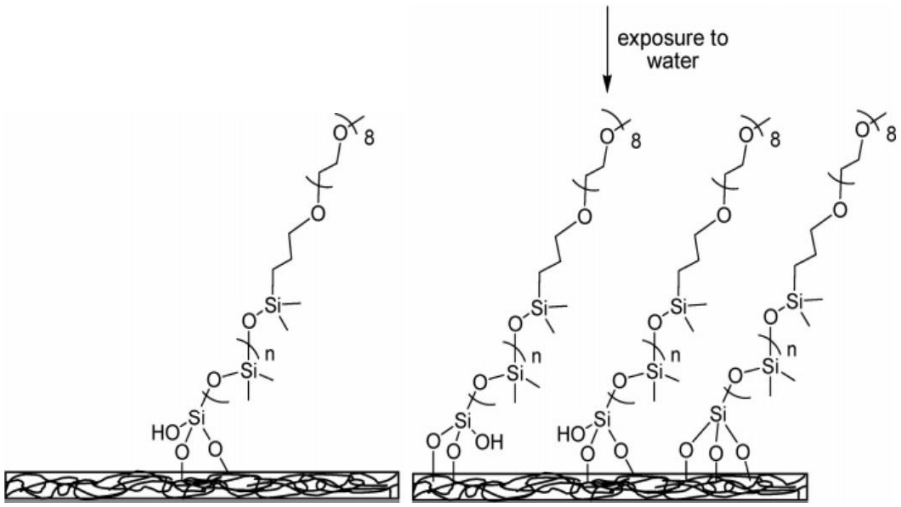

Figure 2: ***Exposition and mobilization of PEO-silane segments (n) to the surface in aqueous environment.*** *Hydrophobicity is reduced as the PEO-silane tethers (n) increased. Adapted with permission from [41]. Copyright 2018 American Chemical Society.*

Hydrophilic properties are further enhanced with amphiphilic polymers. Rufin et,al (2015) produced silicones with higher resistance to protein adsorption by bulk-modification of PDMS with PEO-silane amphiphilic showing an optimum water-driven surface restructuring (Figure 3). The surface-grafted silicon chains of PEO maintained a reduction of the hydrophobicity independent of the aqueous environment. In contrast, when the PEO chains are incorporated, by surface-grafted coating, into the silicon, the hydrophobic properties are recovered similarly as observed in plasma treatment.

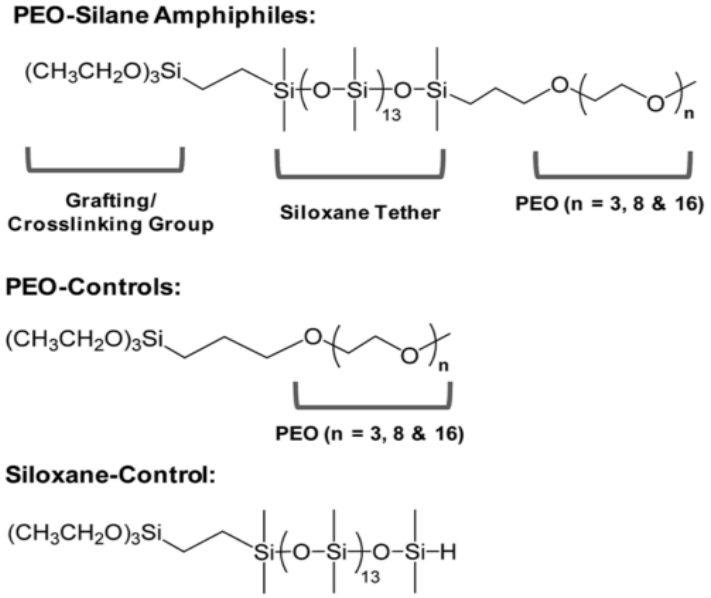

Figure 3. ***Chemical structures of different PEO and Siloxane-control.***
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The impact of PEO-segment length (n=3,8 and 16) in the PDMS bulk was established by comparing PEO: PEO-Silane amphiphilic, PEO-silane and Siloxane-control while keeping the same siloxane tether (*m=13*). The siloxane tethers had a hydrophobic behavior, but the PEO-silane added an amphiphilic property. The water-driven surface restructuring or reduction of hydrophobicity recovery was quantified by water contact angle assay. Results showed an enhanced surface reorganization of the PEO-silane amphiphilic on the silicone surface. The siloxane segment facilitated the migration of PEO tethers to the surface in water contact, producing a higher impact in the reduction of the hydrophobicity (when n=8). The length of

the PEO segments positively corelated with the increased hydrophilicity observed in modified polymers (Figure 4).

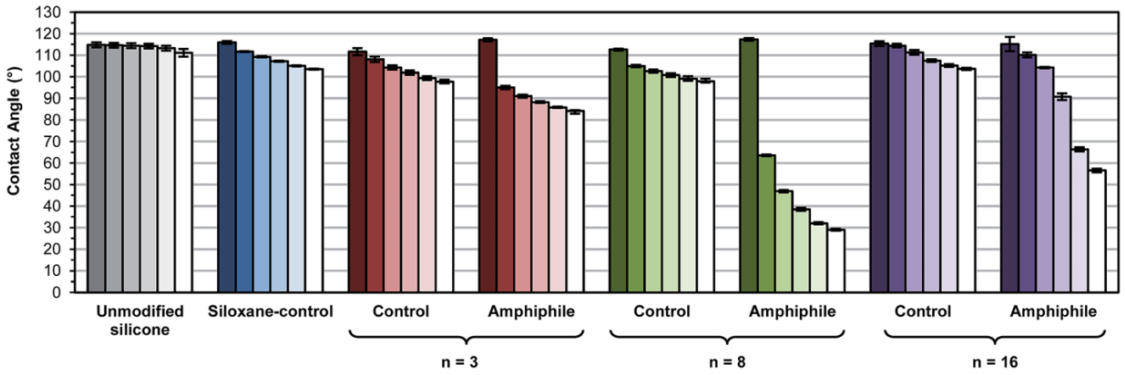

Figure 4. ***Response of the PEO tether on the hydrophobicity surface reduction.*** *Water contact angle measurements at 0s, 15s, 30s, 1min, 2min and 3 min.*
Reprinted with permission from [17]. Copyright 2018 Royal Society of Chemistry.

The segments PEO-silane amphiphilic n=8 and 16, demonstrated a rapid restructuration on the silicone surface reducing it hydrophobicity after 3 minutes exposure to water. This fast mobility is attributed to the hydrophobic nature and flexibility of the siloxane segment which allow the movement of the PEO tether to the surface through the silicone network.[7]

Rufin et.al (2016) in their previous study, determined the PEO-silane amphiphilic (m=13 and n=8) have a higher effect in the reduction of the hydrophobicity but only a single concentration of 50µmol/g of silicone was studied. In regards, five concentrations were evaluated (5, 10, 25, 50 and 100µmol per 1g silicone) to determine the smallest concentration necessary.

Figure 5 showed that the minimum concentration for each different PEO tethers was varied. Tethers of n=8 showed a higher capacity to reduce surface hydrophobicity compared with other PEO tether lengths (n=0 and n=16). The notable hydrophobicity reduction on the silicone water-surface was 10µmol (~2wt%) concentration or higher. The n=8 PEO-SA is considered, with these results, to be a potent surface-modifying additive[6].

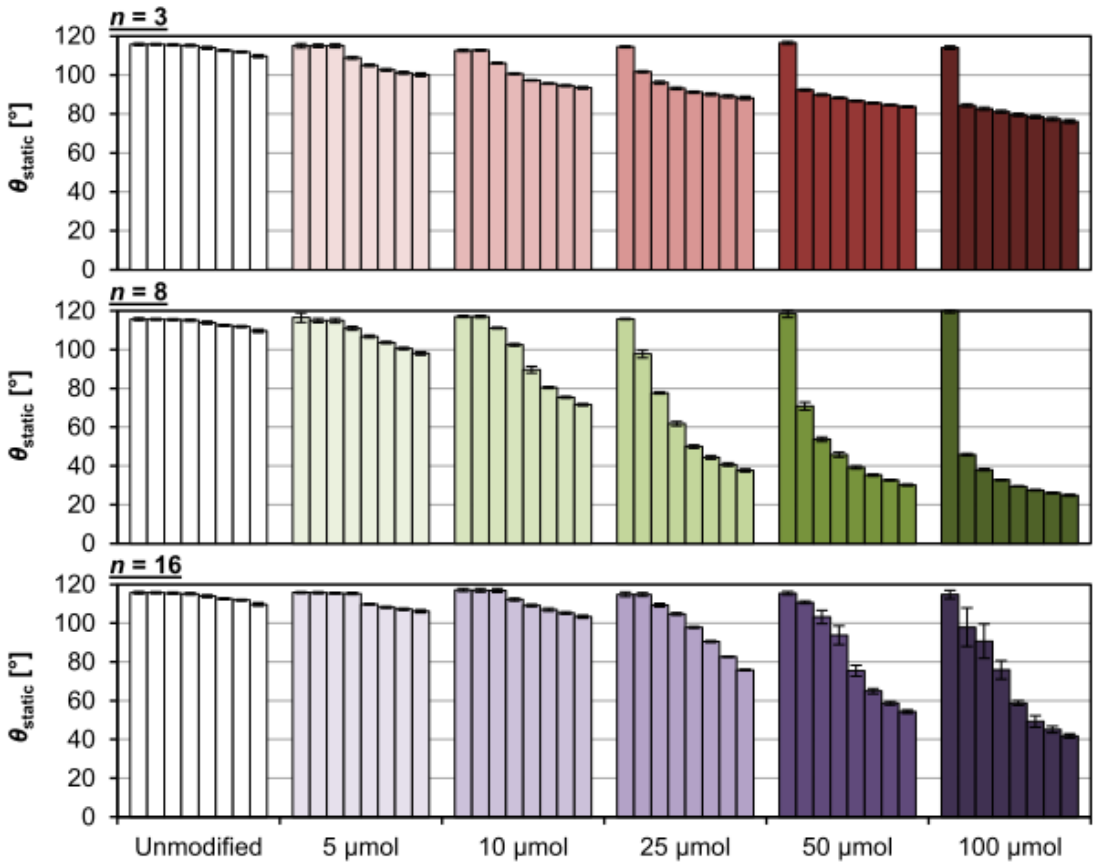

Figure 5. ***Response of the molar concentration of PEO-silane Amphiphilic per 1g silicone.***
Water contact angle measurement at 0s, 15s, 30s, 1 min, 2 min, 3 min, 4 min and 5 min.
Reprinted with permission from [6]. Copyright 2018 Elsevier.

Rufin et.al (2016) evaluated the efficacy of different PEO-(based) amphiphiles surface-modifying additives (SMAs). The difference is respect terms of cross-linkability, siloxane tether length (named "m") and comparing diblock vs triblock chemical architecture. Two di-block polymers, cross-linkable and non-cross-linkable were compared using two different oligodimethylsiloxane (ODMS) tether lengths m=13 and 30 for both.

The authors evaluated the change on the hydrophobicity of silicon surface and water uptake of the different PEO-(based) SMAs, the water contact angle was used to monitor the PEO migration to the silicone surface-water interface. Cross-linkable diblock amphiphile and triblock amphiphile showed the same behavior of reduction (~25°) when the siloxane tether was increased of m=13 to m=30. In contrast, non-cross-linkable diblock amphiphile produced a higher hydrophilicity when m=13 (~10°) against m=30 (~25°), and the cross-linkable diblock

and triblock amphiphile at the same m=13 (~20°). The enhanced water-driven surface of PEO migration of the no cross-linkable vs cross-linkable di-block amphiphile is attributed to the PEO chain mobility that is not limited by covalent attachment to the silicone network. The non-cross-linkable di-block polymer of length m=13 and m=30 produced the highest improvement in hydrophilicity.

However, when water uptake assay was realized (Figure 6), the non-cross-linkable amphiphile m=30 showed the least absorption water (<2wt%) compared to m=13. Increased absorption of water is not optimal for cell culture applications as it can cause osmotic stress. Thus, the non-cross-linkable amphiphile with a longer siloxane tether (m=30) will be used in our studies for reducing absorption of hydrophilic molecules in PDMS.

For all the research discussed above, the focus of this thesis work is the evaluation of the effect of PEO-SA (30,8) in the hydrophobicity of PDMS for cell culture applications and drug assays (Figure 6). [6,7,13].

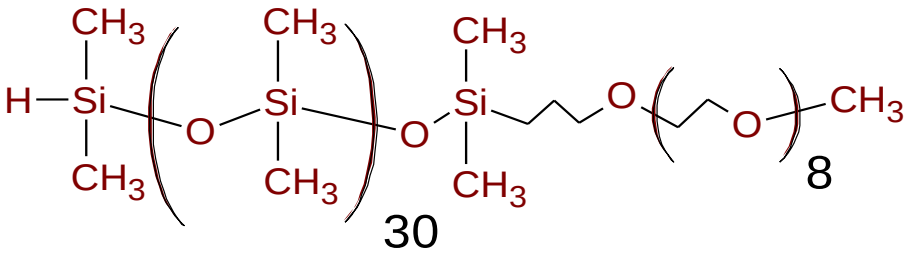

Figure 6. ***Chemical structure of the PEO-silane amphiphile.***

CHAPTER 3 – METHODOLOGY

3.1 *Devices Mold Casting and Oligomer Extraction*

The PEO-SA (MW=2778g/mol)[7]was synthesized by Brian Ngo at the GRUNLAN LAB (TAMU-TX) . The procedure used for polymer synthesis is described as follows.

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[3]. A schematic of the pristine PDMS mold casting process is shown in Figure 7.

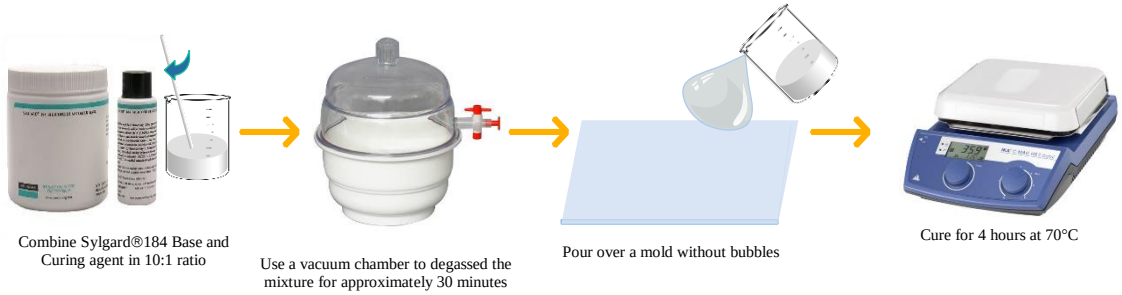

Figure 7. ***Schematic of the pristine PDMS mold casting procedure.***

3.2 *Mold Casting modified PDMS Protocol*

SYLGARD® 184 (1064291, DOW CORNING) base and curing agent were mixed in 10:1 ratio (wt%). For the preparation of modified PDMS, 2wt%, 9wt% and 14wt% of PEO-SA (GRUNLAN LAB, TAMU TX) were added into SYLGARD® 184 mixture using a pre-heated Water Bath (PRECISION™) at 60°C to improve amphiphile solubility since at room temperature PEO-SA (GRUNLAN LAB, TAMU TX) has low solubility in SYLGARD® 184 mixture (1064291, DOW CORNING). The mixture was vigorously stirred for ~5 minutes, until there was a noticeable decrease in viscosity of the mixture. PDMS+PEO-SA mixture was poured over the mold casting surface and heated at 90°C and ~1mbar, using a Vacuum Drying Oven (YAMATO ADP 21). the PDMS+PEO-SA was left for 1hour until fully cured. *Protocol developed by GRUNLAN LAB (TAMU-TX).*

A schematic of the mold casting modified PDMS process is shown in Figure 8.

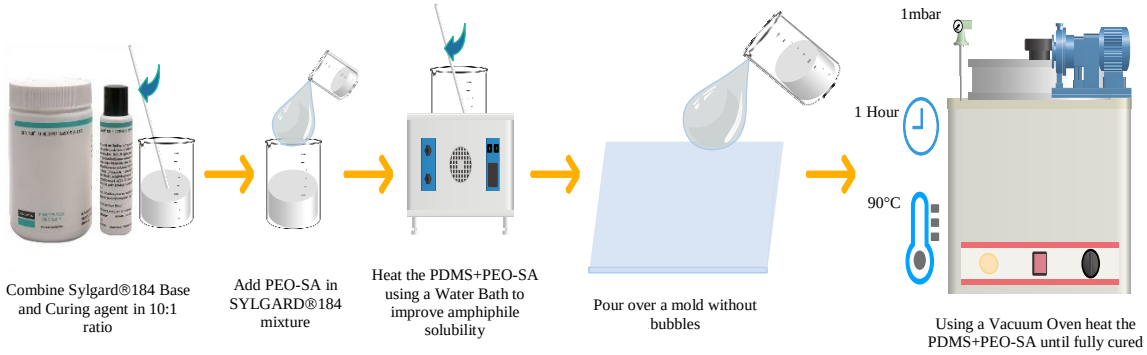

Figure 8. ***Schematic of the modified PDMS (PDMS+PEO-SA) mold casting procedure.***

PDMS and PDMS+PEO-SA were casted into discs and micro wells. The thin discs (16-20mg) were cut from a flat device that was fabricated using Soft Lithography[37] Protocol with a hollow puncher with 5mm diameter. Single polymer discs were weighed in an Analytical Balance (VWR-164AC) before the oligomer extraction was performed.

3.3 ***Uncross-linked Oligomer Extraction***

Single polymer discs (pristine PDMS or PDMS+PEO-SA) were placed in 50 mL centrifuge tube filled with Ethanol 200 proof (459844, SIGMA-ALDRICH)[3]. Twenty single polymer discs were placed in different tubes. Extraction was performed for 1 hour at room temperature. This step removes any un-crosslinked PDMS or PEO-SA oligomer[3]. Then, the single polymer discs were removed and placed in glass petri dish. The glass petri dish was placed inside the Biosafety Cabinet (BAKER COMPANY, INC) for ~1 hour to allow residual ethanol to evaporate (Figure 9). Then, single polymer discs were sterilized via Autoclave Chamber 2340M (TUTTNAUER BRINKMANN) to 121°C x 1 hour, prior to experimental assay. This procedure was realized to all the single polymer discs and microwells before to each detailed experiment below.

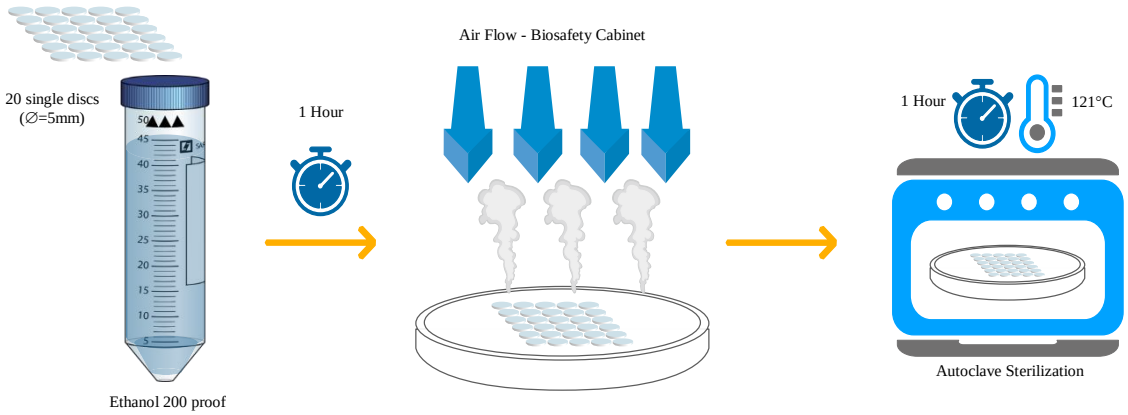

Figure 9. ***Schematic of the oligomer extraction procedure.***

3.4 ***Fourier Transform Infrared Spectroscopy***

To corroborate that the PDMS has been modified by the addition of the PEO-SA, Attenuated Total Reflectance (ATR) FTIR test was performed in the surface and close layers (1.6µm) from the single polymer discs (labeled: pristine PDMS, PDMS+PEO-SA 2wt%, PDMS+PEO-SA 9wt%, PDMS+PEO-SA 14wt%), using an FTIR Spectrometer-Spectrum Two (PerkinElmer). The single polymer discs, with +/-0.5mm thickness, were in direct contact with the ATR diamond crystal and scanned 100 times at room temperature and 90% of gauge pressure; the absorbance measurements in the range of 500-4000cm^{-1} were analyzed using The Unscrambler X v.10.5 (CAMO, Trondheim-Norway) software. A schematic of the measurement of absorbance by ATR-FTIR process is shown in Figure 10.

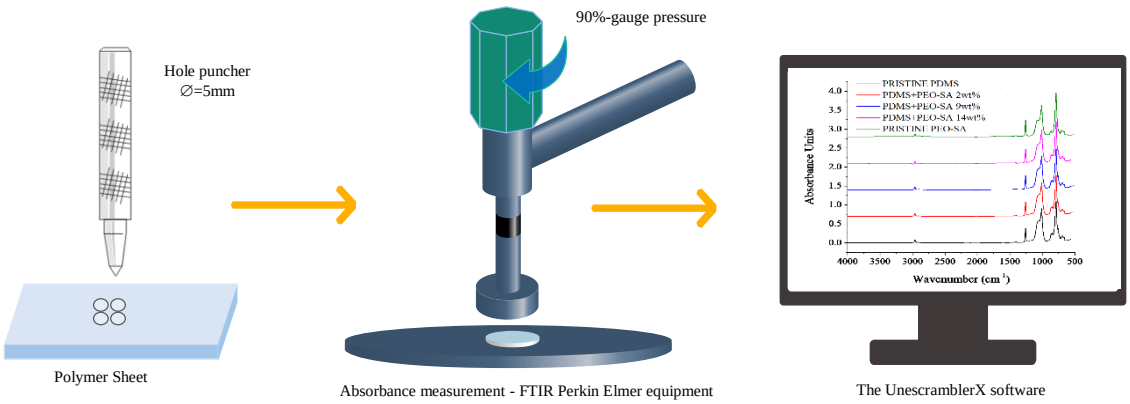

Figure 10. ***Schematic of the measurement of absorbance by ATR- FTIR procedure.***

3.5 ***Water Contact Angle Analysis***

To corroborate incorporation of PEO-SA into PDMS the surface contact angle was measured to confirm a reduction of hydrophobicity. A surface is considered hydrophobic when the surface angle is greater than 90° and is considered hydrophilic when the angle is less than 90° [41].

The single PDMS disc was treated and modified by plasma treatment. The PDMS surface was modified when exposed to electric field for 5 minutes using the CORONA PLASMA instrument (BD20-AC) [42].

The contact angle of a liquid water interface was measured on the surface of single polymer discs (labeled: pristine PDMS, PDMS+PEO-SA 2wt%, PDMS+PEO-SA 9wt%, PDMS+PEO-SA 14wt%) at different time intervals (0 sec-3 months). A volume of 8 µL drop of sterile water was placed on the surface of Pristine PDMS, PDMS plasma bonding, PDMS+PEO-SA (2wt%, 9wt% and 14wt%) and PS. The benchtop in which the single polymer discs were placed must to be flat (~0º) and aligned with the HDMI Digital Microscope (CRENOVA). The surface contact angle obtained from the images were measured using ImageJ v.1.50i software (NIH, MD-USA).

A schematic of the water contact analysis is shown in Figure 11.

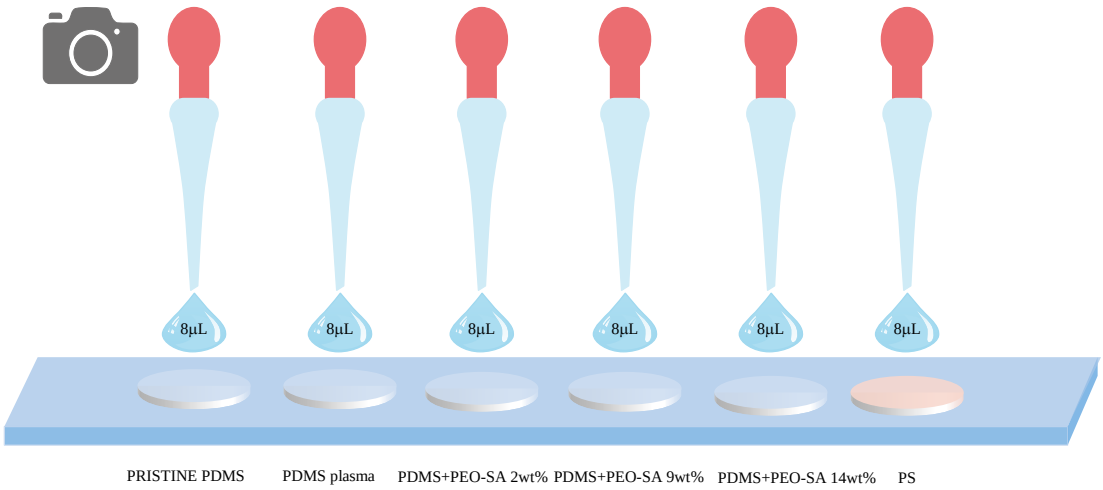

Figure 11. ***Schematic of the water contact angle analysis.***

3.6 ***Optical Transparency Assay***

To evaluate the optical transparency of the modified PDMS an absorbance assay was done. Absorbance was compared with the pristine PDMS as control. Single polymer discs of pristine PDMS and the PDMS+PEO-SA 2wt%, 9wt%, and 14wt% were placed into 96-well plate and analyzed in UV/VIS Spectra Multiplate Reader (INFINITE 200 PRO, TECAN) at 600nm wavelength[43].

The optical transparency was calculated by the equation:

$$T = \frac{Abs600}{x},$$

T= transparency

Abs600= Absorbance at 600nm wavelength

X= polymer thickness (mm)

The value T indicate: T >, lower transparency and higher opacity.

T<, higher transparency and lower opacity.

A schematic of the optical transparency assay is shown in Figure 12.

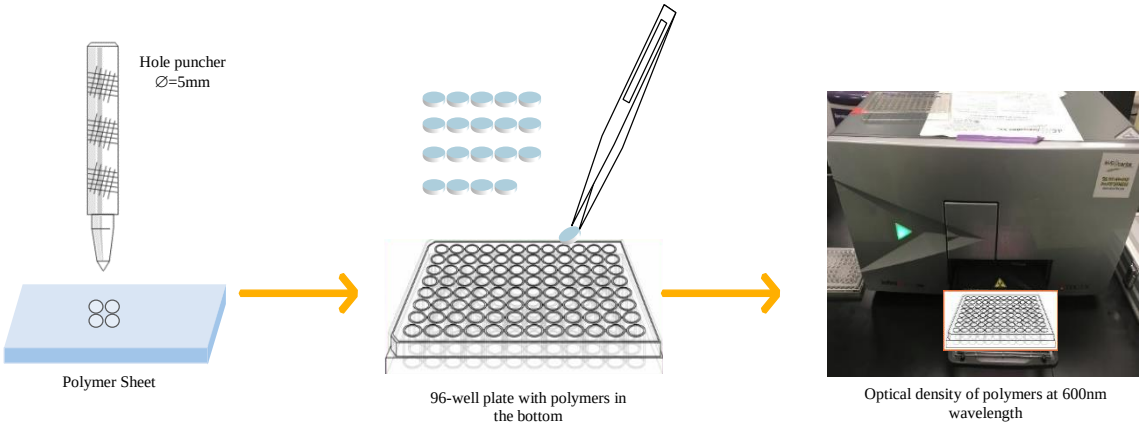

Figure 12. ***Schematic of the optical transparency assay.***

3.7 ***Hydrophobic/Hydrophilic Absorption***

For the evaluation of hydrophobic/hydrophilic absorption of small molecules into polymers, two molecules were evaluated:

Table 2. ***Molecules placed in microwells for the qualitative study of absorption.***

Molecules	*Size*	*Description*
NILE RED (19123, SIGMA-ALDRICH)	*~320 Da*	*Lipophilic fluorophore*
TRYPAN BLUE (T8154, SIGMA-ALDRICH)	*~960 Da*	*Hydrophilic cell stain*

Nile Red and Trypan Blue were selected for their molecular size (<1KDa) and fluorescence/colorimetric property to optically monitor absorption into the PDMS substrates[14].

Nile Red is a lipophilic fluorophore with high selectivity and sensitivity that can dissolve in methanol, ethanol or water. In water the Nile Red's fluorescence intensity is weak, conversely, when a hydrophobic-rich environment is present the fluorescence intensity is strong. The PDMS to has siloxane in it composition, mimic this environment[14,44]. Instead, Trypan Blue is a large acid dye hydrophilic used to detect dead cells in viability assay, and as a model hydrophilic drug in studies of drug delivery systems for liver cancer therapy[45]. This allowed us to evaluate if the reduction in hydrophobicity increases the hydrophilicity to such an extent that it becomes a trouble.

Absorption of 1mM Nile Red was visually monitored to predict behavior of small hydrophobic drugs. Similarly, absorption of 1mM Trypan Blue was visually monitored to predict behavior of small hydrophilic drugs. Qualitative analysis was done by comparing the stained surface area obtains for each polymer: Pristine PDMS and PDMS+PEO-SA at 2wt%, 9wt% and 14wt%. Images of the bottom of a microwell made of each polymer were taken in time intervals of 15 seconds, 5 minutes and 30 minutes for Nile Red, and 0, 20 min and 40min for Trypan Blue using an inverted fluorescence microscope (PRO282B, MOTIC).

The microwells (~2mm diameter) were made with a hole puncher, placed on a microscopy slide with a volume of 3µL (hydrophobic/hydrophilic molecule). A schematic of the Nile Red/Trypan Blue hydrophobic/hydrophilic absorption process is shown in Figure 13.

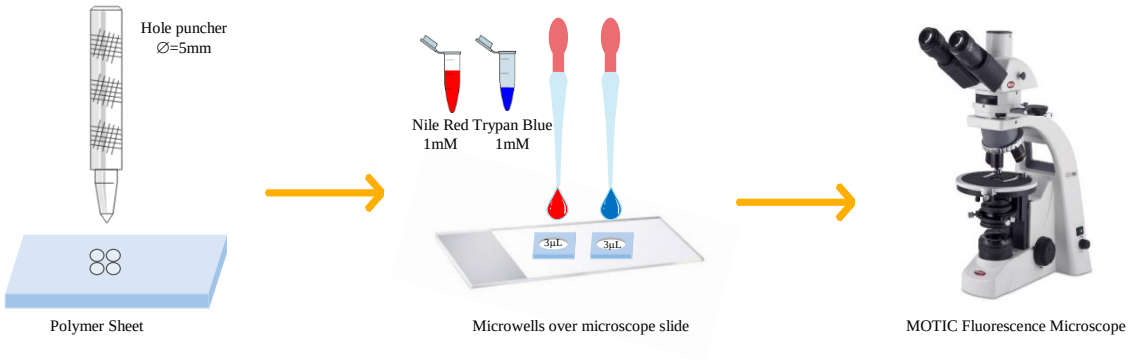

Figure 13. ***Schematic of molecules absorption procedure.***

3.8 *Cell Culture*

The following cell lines were used to evaluate cell behavior and biocompatibility of PDMS-PEO-SA substrates:

Table 3. ***Cells Lines obtained from the American Type Culture Collection (ATCC)***[46]

Cells	*Type*	*Size*	*Doubling time*	*Sensitive to:*
MCF-7	*Human BREAST CANCER*	*12µm*	*30h*	*β-ESTRADIOL*
MDA-MB-231	*Human BREAST CANCER*	*16µm*	*28h*	*DOX.HCl*
MDA-MB-468	*Human BREAST CANCER*	*14µm*	*30h*	*DOX.HCl*
NIH-3T3	*Mouse FIBROBLAST*	*15µm*	*23h*	--

NIH-3T3 were selected as baseline cell line model of normal cells. MDA-MB-231 and MDA-MB-468 were selected as representative cell line for tumor cells and, also, as a sensor cell line for effective concentration of Doxorubicin Hydrochloride (DOX.HCL), a hydrophilic drug. If DOX.HCL is absorbed into the PDMS+PEO-SA substrate, cell toxicity will not be affected at the effective dose used in tissue culture plastic. MCF-7 cells were selected as a sensor cell line for effective concentration of estrogen. As MCF-7 cells are highly dependent on estrogen concentration for cell growth[3], we expect proliferation rates to be reduced if estrogen is sequestered into PDMS substrates.

All cell lines were maintained 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 100-unit Penicillin/ml, and 100 µg Streptomycin/ml (P4333, SIGMA-ALDRICH). Cells were culture inside an CO_2 Incubator 2200 (VWR) maintained at 37°C in 5% CO_2[32,47]. To dissociate adherent cells from the flask 0.25% (v/v) Trypsin-EDTA solution (T4049, SIGMA-ALDRICH) was used.

For evaluation of cell behavior using estrogen-dependent MCF-7 cells, confluent cell monolayers were washed three times with PBS 1X and incubated at 37°C in 5% CO_2 overnight in a flask containing no-phenol red cell culture medium composed by phenol-free DMEM (D1145, SIGMA-ALDRICH), supplemented with 10% (v/v) Fetal Bovine Serum Charcoal Stripped (F6765, SIGMA-ALDRICH) and 100-unit Penicillin/ml, and 100 µg Streptomycin/ml (P4333, SIGMA-ALDRICH). To dissociate adherent cells from the flask phenol red-free 0.05% (v/v) Trypsin-EDTA solution (59418C, SIGMA-ALDRICH) is used. A schematic of cell culture process is shown in Figure 14.

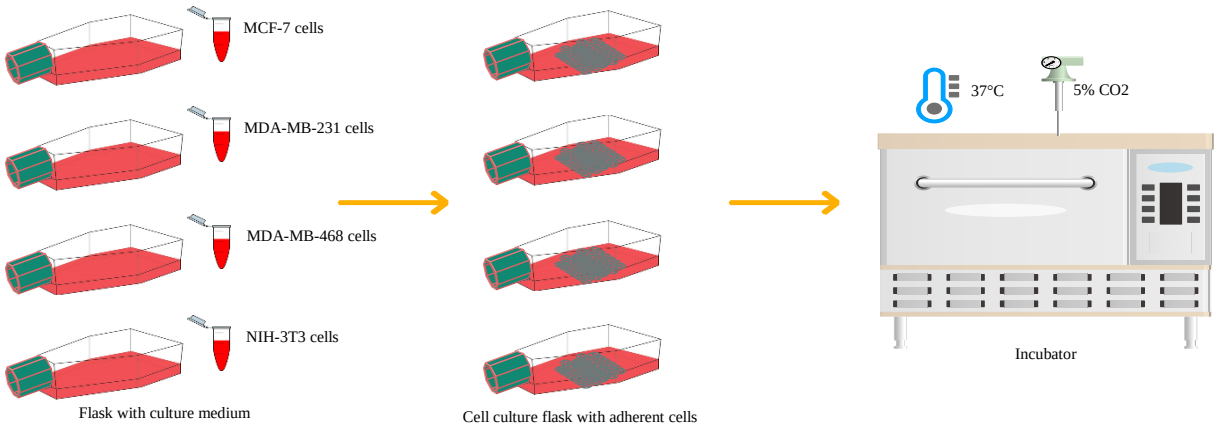

Figure 14. ***Schematic of cell culture procedure.***

Viable cells were counted using Cellometer Vision CBA Image Cytometer (NEXCELOM). The viability assay for cell concentration used 0.4% Trypan Blue solution (T8145, SIGMA-ALDRICH), to stain late apoptotic cells. A 20 uL sample containing detached cells were taken and combined with 20 µL Trypan Blue in a sample tube and mixed gently. A 20 µL of sample was loaded into a disposable counting chamber and then inserted the chamber in the Cellometer (Vision CBA Cellometer).

The output "Live cell count" is generated instantly. For experimentation, a concentration of 30,000 cells/well was required and seeded in 96-wells plate. A schematic of experimental cell seeding process is shown in Figure 15.

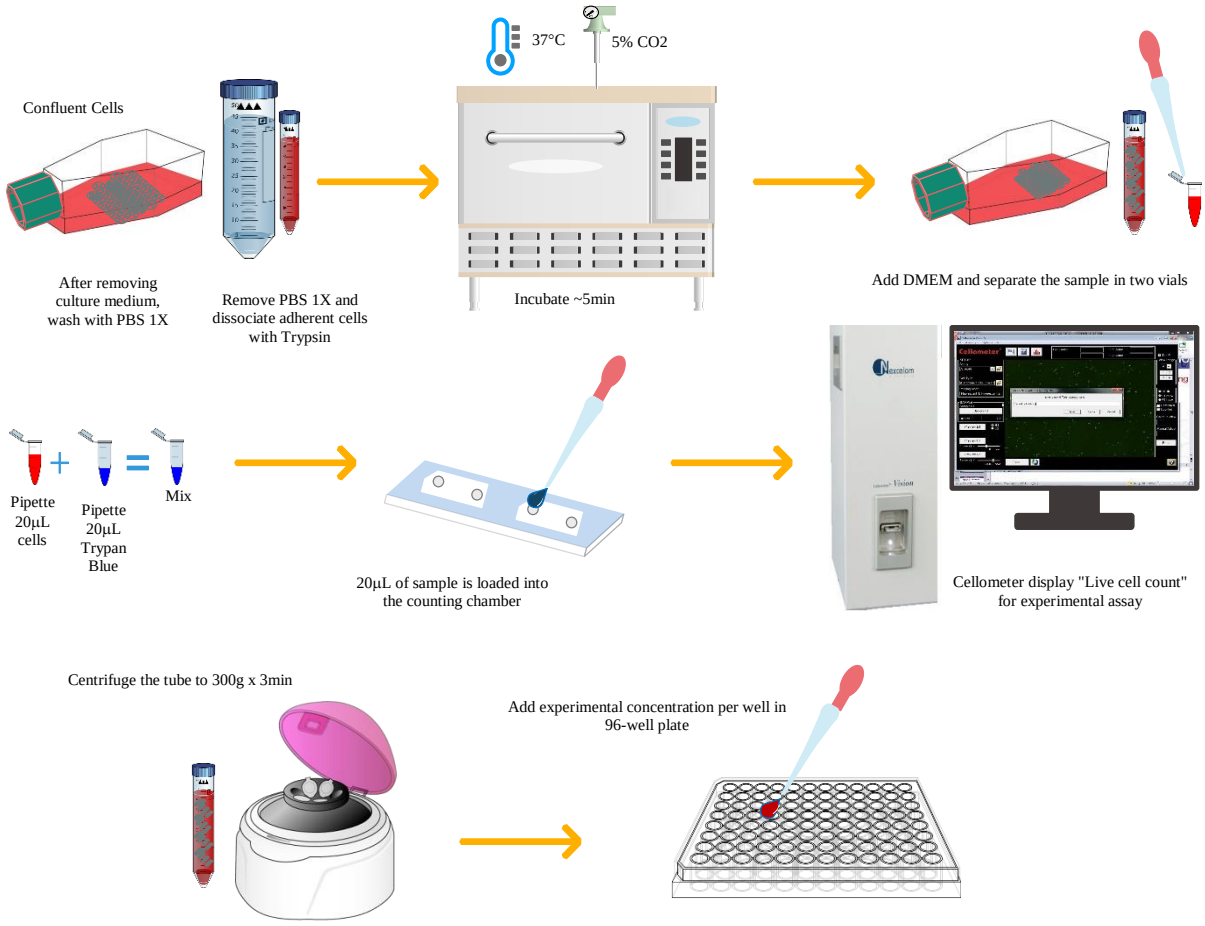

Figure 15. ***Schematic of experimental cell seeding procedure.***

3.9 ***Biocompatibility and absorption assay.***

To determine the biocompatibility of the PDMS+PEO-SA substrates, cell viability, photo-morphology, and cell proliferation were evaluated in MCF7 cells lines (culture medium+/-phenol red), MDA-MB-231, MDA-MB-468 and NIH-3T3.

Drug absorption impact of PDMS+PEO-SA on cell behavior were evaluated in MCF7 cell line (culture medium+/- phenol red), MDA-MB-231, MDA-MB-468 and NIH-3T3. Only MCF-7 Cell line was evaluated in response to β -estradiol using no-phenol red culture medium. Cell viability was quantified based on the detection of the cellular metabolic activities[48], and proliferation-detection was performed by EDU incorporation into DNA cells during DNA replication (S phase of active synthesis)[49]. Respect the absorption assay, one drug (Doxorubicin

Hydrochloride), frequently used in cancer treatment was used to evaluate the impact on viability in several cell lines.

Adherents cells were seeded in 96-well plate, with a concentration of 30000 cells/well and a volume of 200µL. The cells seeded were incubated for 24 hours at 37°C in 5% CO_2 overnight. We worked with five labeled conditions: 1) Tissue Culture Plastic, TCP (no polymer), used as a blank. 2) PRISTINE PDMS. 3) PDMS + PEO-SA 2wt%. 4) PDMS + PEO-SA 9wt%. 5) PDMS + PEO-SA 14wt%.

After 24-hours, culture medium was removed and replaced with fresh culture medium +/- single polymer discs. Polymer discs remained in the surface of the culture medium inside the culture wells. The cells were incubated for 48 hours at 37°C in 5% CO_2.

a) Viability assay

The metabolic activity was measured by XTT ((sodium 3′-[1-[(phenylamino)-carbony]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate) absorbance. XTT (X4626, SIGMA-ALDRICH) is a tetrazolium salt widely used in cell biological assays as cytotoxicity[48] and apoptosis[50]. The XTT assay is a good method to measure drug sensitivity in cancer cell lines, by direct proportionality of XTT reduction and cell concentration, but a detectable signal reduction is slow[51]. The reduction of XTT of yellow to orange, originated by the mitochondrial dehydrogenase enzymes present in living cells[52]. Menadione (02102259, MP BIOMEDICALS), an electron-coupling agent, was added to XTT (X4626, SIGMA-ALDRICH) to accelerate the incubation time[53]. This assay allowed us to evaluate UV/VIS absorption at 465nm wavelength[50].

After removing the single polymers discs and the culture medium, fresh culture medium with 3mM XTT-menadione (200µL culture medium/100 µL XTT-menadione) was added and incubated for 2-4 hours at 37°C in 5% CO_2. Then, 100µL of culture medium/XTT-menadione was transfer in a new 96-well plate for the absorbance measurements in UV/VIS Spectra Multiplate Reader (INFINITE 200 PRO, TECAN) [50] at 465nm wavelength. This measure was corrected at 600nm wavelength. Brightfield images to compare cell morphologies were obtained prior to the cell toxicity assay using ZOE™ Fluorescent Cell Imager (Bio-Rad). A schematic of the viability assay is shown in Figure 16.

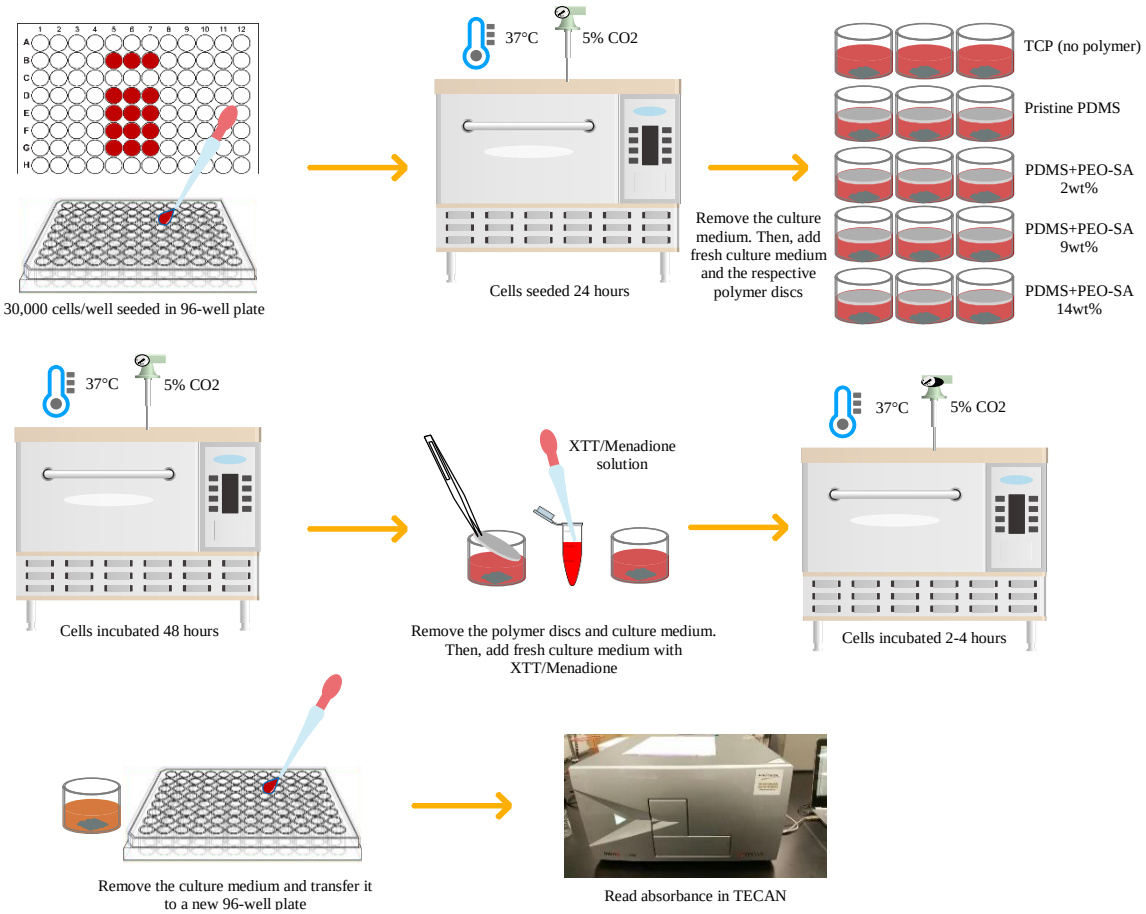

Figure 16. ***Schematic of the viability assay.***

b) Absorption assay

For the evaluation of absorption of small drugs (1<KDa) into polymers, one hydrophilic drug and one steroid hormone with size closed to Nile Red were evaluated (Table 4).

Table 4. ***Small drugs evaluated by absorption assay.***

Molecules	*Size*	*Description*
β-ESTRADIOL (E2758, SIGMA-ALDRICH)	*~273 Da*	*Hydrophobic hormone*
DOXORUBICIN HYDROCHLORIDE (D1515, SIGMA-ALDRICH)	*~580 Da*	*Hydrophilic drug*

Doxorubicin Hydrochloride (DOX.HCL) is a hydrophilic hydrochloride salt of doxorubicin[54], that induces apoptosis in breast cancer cells by inhibition of the respiratory mitochondria[55].β-Estradiol (E2), is an estrogen steroid hormone responsible of the growth of epithelial cells and supports expansion of some tumors in the mammary gland [56]. Previous studies have documented the estrogen-dependent growth behavior of MCF-7 cell line and highlighted a relationship among estrogen sequestration into PDMS and the decreased impact on luciferase signal over several concentrations[3].

For the evaluation of estrogen response on viability, MCF-7 cells were seeded in no-phenol red culture medium. For the study of DOX.HCL response on viability, MCF-7, MDA-MB-231 and MDA-MB-468 cells were seeded in phenol red culture medium. After 24 hours, culture medium +/- phenol red is replaced with culture medium +/- phenol red supplemented with 4µM DOX.HCL (Figure 17) and +/-0.1nM estrogen prepared from β-Estradiol (E2758, SIGMA-ALDRICH) in Ethanol 200 proof (459844, SIGMA-ALDRICH) +/- single polymer discs, then, viability assay is performed.

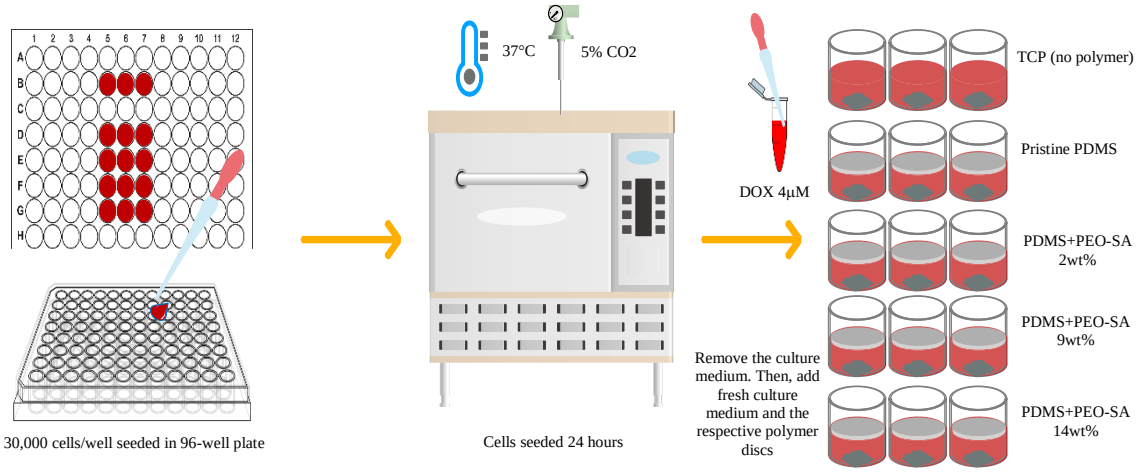

Figure 17. ***Schematic of DOX.HCL absorption into the PDMS substrates procedure.***

c) Proliferation assay

The DNA of proliferating cells were label with EdU using the Click-iT EdU Alexa Fluor 594 Image Kit (C10425, INVITROGEN) according to manufacturer's recommendation. Cell images were taken using the ZOE™ Fluorescent Cell Imager (Bio-Rad). Cell proliferation was quantified based on total nuclear cell counts and fluorescent labeling of cells that synthesized

new DNA[57] using ImageJ v.1.50i software (NIH, MD-USA) particle counting software[58]. A schematic of the proliferation assay is shown in Figure 18.

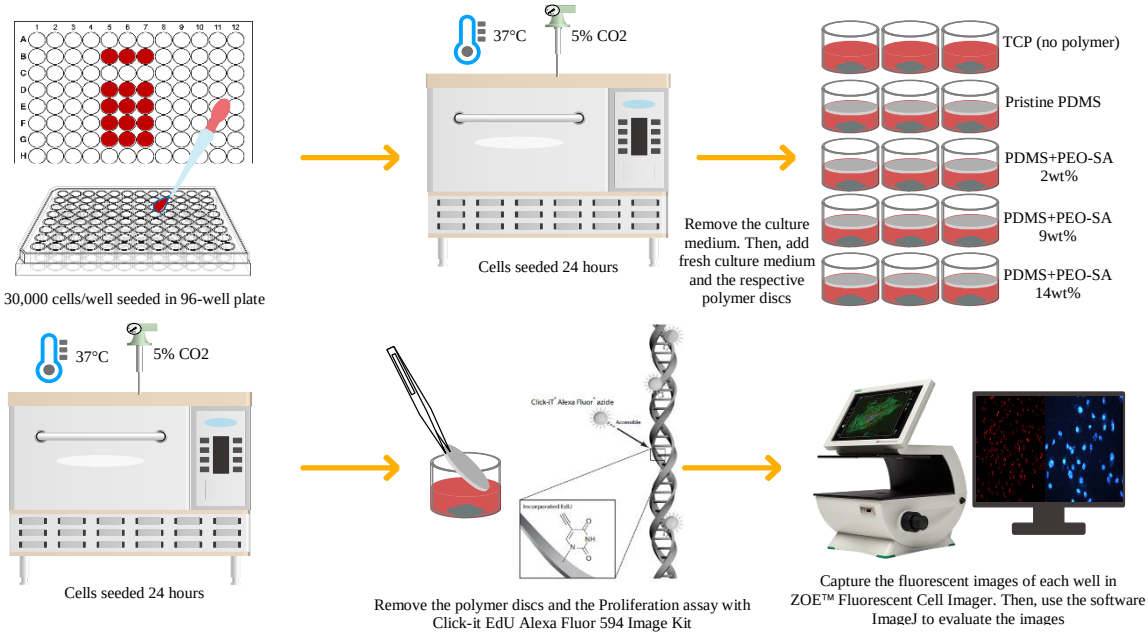

Figure 18. ***Schematic of the proliferation assay.***

3.10 ***Statistical Analysis***

The experimental data was analyzed using The Unscrambler X v.10.5 (CAMO, Trondheim-Norway) software and Origin Pro8.6 (ORIGINLAB, MA-USA) software for the FTIR assay and Minitab 17 (MINITAB Inc. PA-USA) Statistical Software for the analysis of all the remaining experiments. The data were compared using One-way ANOVA post-hoc Dunnett Test with 95% of confidence.

CHAPTER 4 - RESULTS

To evaluate the reduction of drug absorption, the PRISTINE PDMS was cured in contact with PEO-SA. During the preparation of the PDMS, the base and curing agent were crosslinked through a Hydrosililation mechanism (Figure 1). Based in this mechanism a possible polymeric alloy of PEO-SA in the siloxane backbone of PDMS[8,59,60] could have been achieved following the arrangement shown in the Figure 19 or a simple polymeric blending. The crosslinking process was catalyzed using platinum in the presence of heat[61]. PDMS+PEO-SA concentrations of 2wt%, 9wt% and 14% were evaluated.

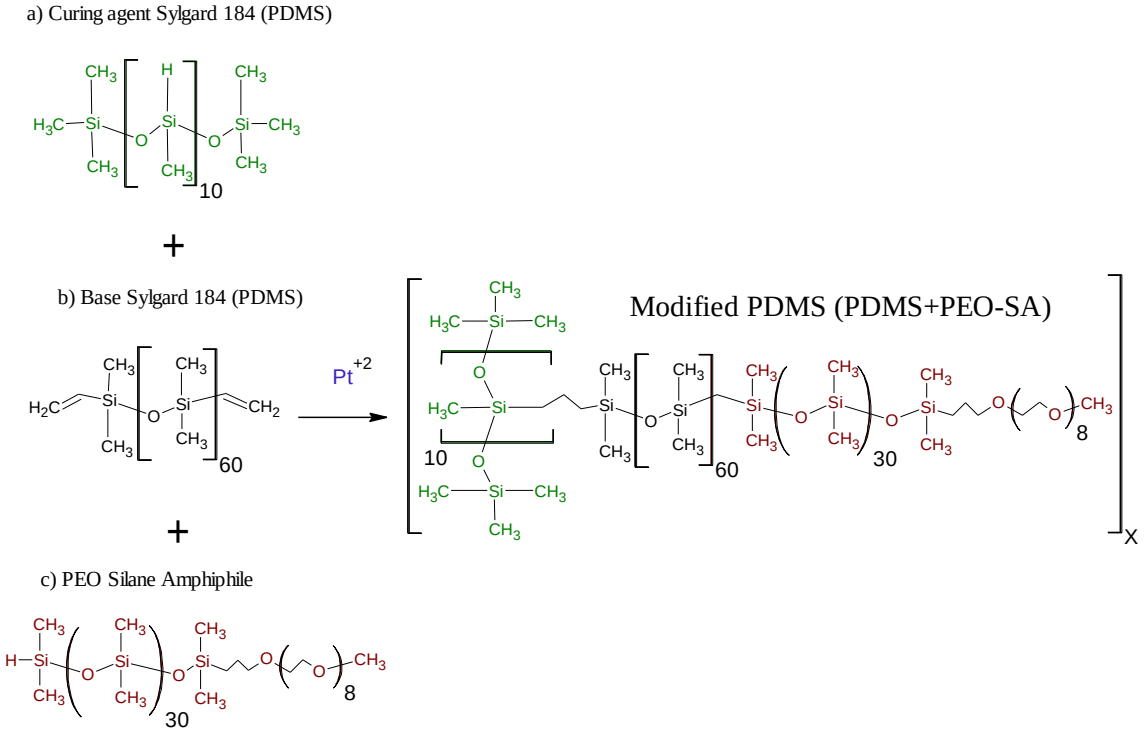

Figure 19. ***Hydrosilylation mechanism proposed in the preparation of PDMS+PEO-SA.***

The 14wt% concentration proved to be the maximum amount of PEO-SA that must be incorporated in silicone polymers without altering its optical properties[7]. The 2wt% concentration appear to be the minimum concentration required to achieve a reduction in PDMS hydrophobicity[6]. Finally, the 9wt% concentration was chosen to evaluate the absorption reduction at an ~intermediate value and calculate a relation between both limits.

Since the PDMS is a polymer biocompatible with poor or no cellular adhesion, attributed to its hydrophobicity as a main factor[62,63], the PRISTINE PDMS and PDMS+PEO-SA (2wt%, 9wt% and 14wt%) were casted into discs and microwells, weighed and oligomer-extracted were used for experimental purposes. Polymer sheets were stored in sealed plastic bags to avoid humidity, dust or contamination (Figure 20).

Figure 20. ***PDMS substrates sheet after the mold casting procedure.***

The present thesis was focused in 4 specifics aims completed and discussed in the next section.

4.1 ***Aim 1. To confirm PEO-SA incorporation into PDMS substrates***

To fulfill this objective, two experiments were carried out: a) Fourier Transform Infrared Spectroscopy and b) Water contact angle analysis.

a) Fourier Transform Infrared Spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was the method used to determine qualitatively the surface modification of PDMS by incorporation of PEO-SA to 2wt%, 9wt% and 14wt%. Five polymer discs with similar diameter (~5mm), weight (~17mg) and thickness (0.50 mm) were taken from different places in the polymer sheets and were measured in separate. Additional was made the measurement of PRISTINE PEO-SA, to observe some possible tendency in the increase of the PEO-SA concentration when was added into the PDMS to different concentration.

The measurements were made in a FTIR Spectrometer-Spectrum Two (PerkinElmer) (Figure 21) and analyzed in The Unscrambler X v.10.5 (CAMO, Trondheim-Norway) software.

Figure 21. ***Perkin Elmer FTIR Spectrum Two located in CENAC Lab-UPRM.***

The data was evaluated by Principal Component Analysis (PCA). This statistical tool allows us to perform an exploratory analysis of the collected data, realizing a dimensionalization and capturing patterns in the data of each variable. PCA displaying the similar patterns of the observation and plot these as points in a map (score plot)[64,65].

A PCA in absorbance units using the 4000-500 cm^{-1} spectral range was developed to find relationships between the variables. Figure 22 shows the score plot. Figure 22.a shows the score plot of the first two Principal Components (PC). The first component (PC-1) explains the greater variability present in the data (90%). This first component shows how changes in the concentration of PEO-SA changed the PDMS. As the concentration of PEO-SA increases in the PDMS, the score plot increases through the first component from 0wt% (PRISTINE PDMS) to 100wt% (PRISTINE PEO-SA). The score plot also shows that the samples that differ most are those that are at the extremes, which represent the pure polymer either PRISTINE PDMS or PRISTINE PEO-SA. The second principal component (PC-2) explains 8% of the data variability. This component can be attributed to difference between thickness, optical transparency or other physical alteration of the samples.

Figure 22.b show the score plot of a PCA performed to evaluate only the PDMS modification without regarding the PRISTINE PEO-SA. The score plot show a tendency with

respect to increase in concentration of PEO-SA in the first principal component. The first and second principal components explain 95 and 2% of the variability of the data. Principal Component Analysis allowed us to find patterns in the data influenced significantly by the modification of the PDMS.

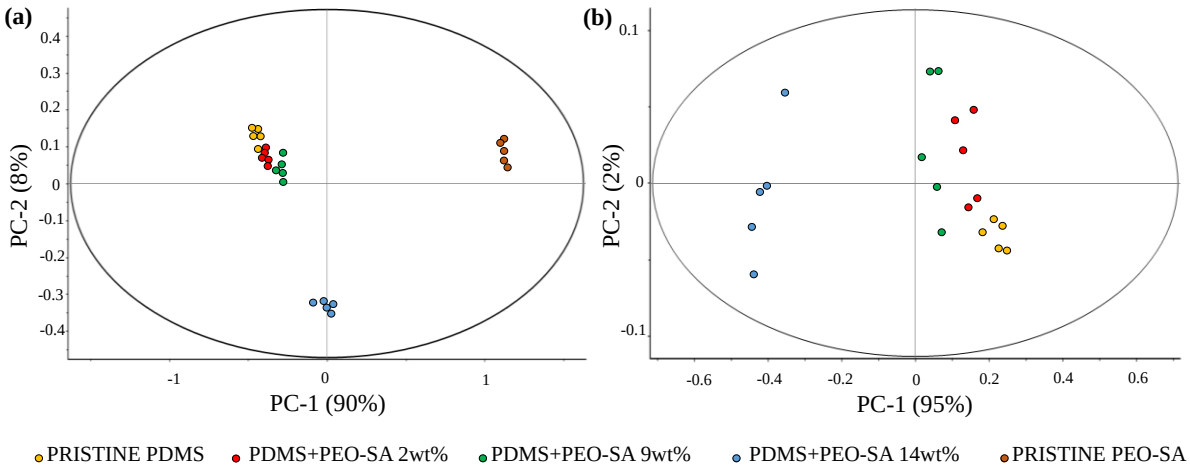

Figure 22. ***PDMS modification FTIR scatter plots.*** *a) PCA plot for each FTIR measurements produced by PRISTINE PDMS, PDMS+PEO-SA 2wt%, 9wt%, 14wt% and PRISTINE PEO-SA. b) PCA plot for each FTIR measurements produced by PRISTINE PDMS and PDMS+PEO-SA 2wt%, 9wt%, 14wt%. Five replicates per polymer were performed to 95% confidence interval and graphed in points.*

FTIR spectroscopy was used to demonstrate the incorporation of PEO-SA into the PDMS by chemical functionalities analysis. Peaks assignment were performed at 4000-500 cm^{-1} spectral range[23]. FTIR spectra of PRISTINE PDMS, PDMS+PEO-SA 2wt%, PDMS+PEO-SA 9wt%, PDMS+PEO-SA 14wt% and PRISTINE PEO-SA are shown in the Figure 23.a. The representative spectra of the PDMS substrates studied shows IR absorption bands similar to each other, this is explained because PRISTINE PDMS and PEO-SA have the same functional groups in their polymeric structure[6,7]. The identification of the representative functional group for PRISTINE PDMS and PEO-SA are shown in Figure 23.b. To the PRISTINE PDMS, vibrational bands around: ~2990cm^{-1} correspond to asymmetric -CH_3, stretching in Si-CH_3, ~1240cm^{-1} correspond to symmetric -CH_3 deformation in Si-CH_3, ~1120-1000 cm^{-1} correspond to Si-O-Si stretching, ~840-770 cm^{-1} correspond to -CH_3 rocking and Si-C stretching in Si-CH_3[23,66]. The PRISTINE PEO-SA IR vibrational bands are the same respect to PRISTINE PDMS, but around ~915-865cm^{-1} harmonic bands of C-C-O appear in the spectrum[67].

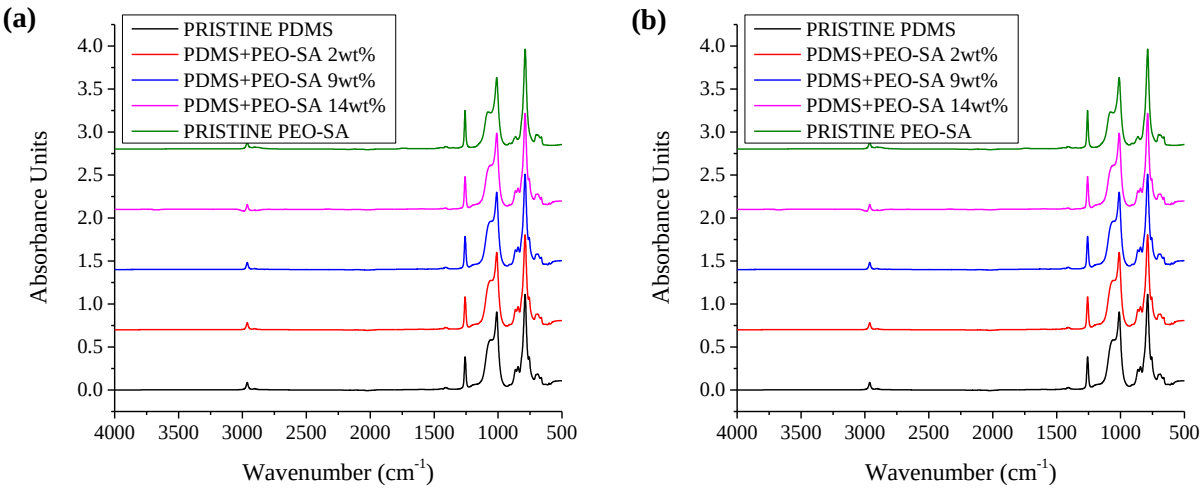

Figure 23. ***FTIR spectra of PDMS modification.*** *a) FTIR spectra of PRISTINE PDMS, PDMS+PEO-SA 2wt%, 9wt%, 14wt% and PRSITINE PEO-SA. b) FTIR spectra of PRISTINE PDMS and PRISTINE PEO-SA with characteristic peaks. Data represent the average of 5 replicates per polymers.*

The analysis of the modified PDMS was complicated since absorption peaks at ~915-865cm^{-1} overlap with other. The presence of PEO-SA in the modified PDMS spectra were evaluated by the slightly signal produced by the characteristic C-C-O group only present in compounds modified with PEO, Second Derivative Spectroscopy data preprocessing, which, enhances the measurement by separation of overlapping peaks was used[68].

Table 5 summarizes the IR vibrational bands obtained by the FTIR spectra of PRISTINE PDMS and PRISTINE PEO-SA.

Table 5. ***Assignment of IR spectra of PRISTINE PDMS and 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

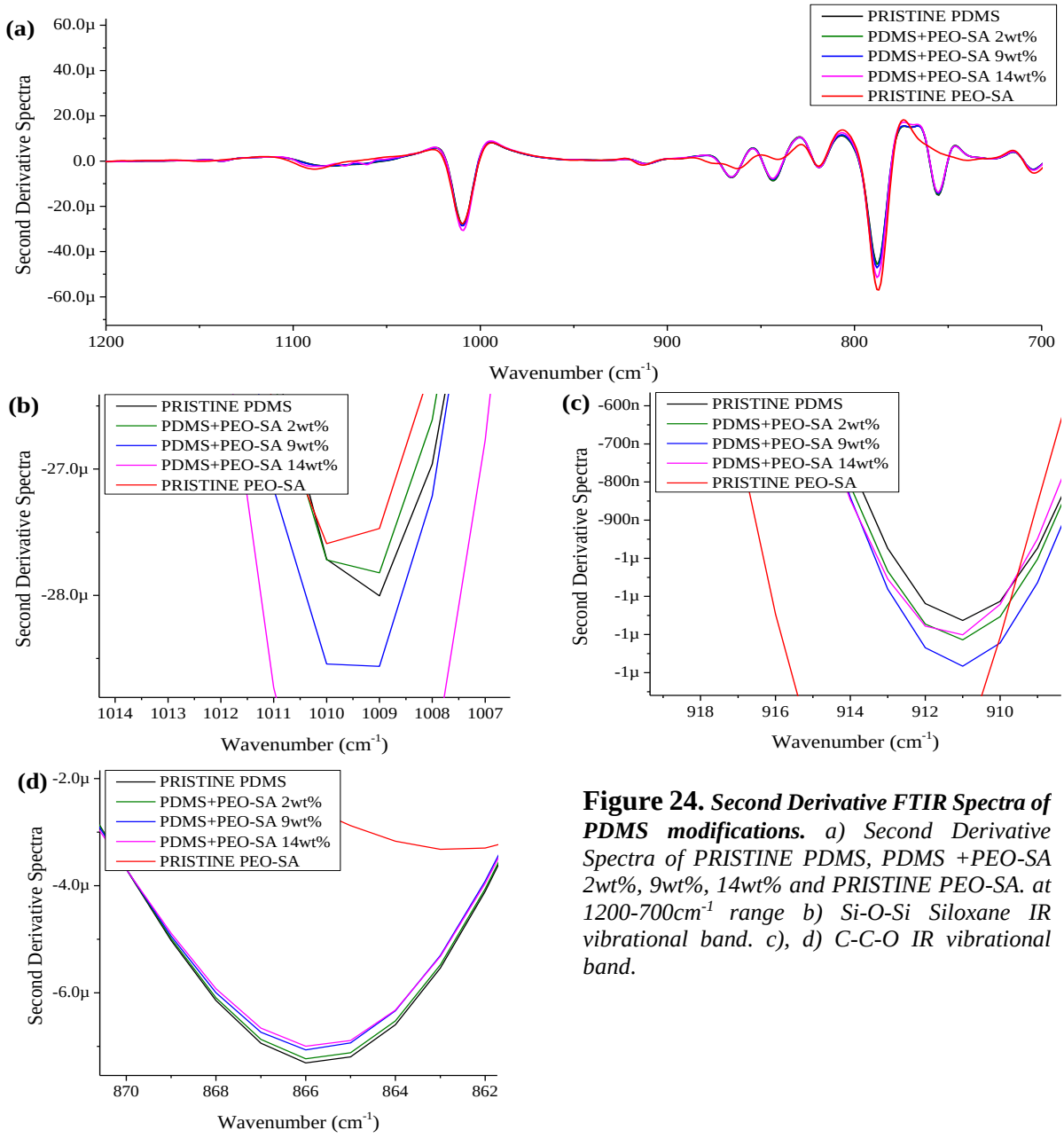

Figure 24. ***Second Derivative FTIR Spectra of PDMS modifications.*** *a) Second Derivative Spectra of PRISTINE PDMS, PDMS +PEO-SA 2wt%, 9wt%, 14wt% and PRISTINE PEO-SA. at 1200-700cm^{-1} range b) Si-O-Si Siloxane IR vibrational band. c), d) C-C-O IR vibrational band.*

The data was normalized and Second Derivative spectrum was calculated for each PDMS substrate using Savisky-Golay algorithm with 7 smoothing points,[68] at 1200-700cm^{-1} range for the evaluation of the main composition of PRISTINE PDMS and PEO-SA at 1010 cm^{-1} (Si-O-Si Siloxane), 915 and 865cm^{-1} (C-O-C group) IR vibrational bands (Figure 24.a).

In figure 24.b the Si-O-Si IR vibrational band was evaluated (1010cm^{-1}). The PRISTINE PEO-SA has Si-O-Si groups in lower concentration compared to the rest of the

polymers, in which, an apparent increase was visualized having a relationship with the increase in the concentration of PEO-SA added into the PDMS. This probable increase in the Siloxane group (Si-O-Si) could be attributed to the addition of PEO-SA by the suggested mechanism showed in the Figure 19.

When we evaluated the C-C-O group, the weak and overlap peak characteristic of PEO-SA was slightly amplified using the Second Derivative tool, in Figure 24.c, an apparent tendency of concentration pattern could be observed in the absorbance peaks of the PDMS substrates, showing that the lowest peak is the PRISTINE PDMS, and the highest peak is PRISTINE PEO-SA, which would be the expected because the C-C-O group is only present in compounds modified with PEO-SA[67].

When the absorbance peaks were compared in Figure 24.d, the absorption peaks showed a slightest increase proportional to the increase in PEO-SA concentration in the surface of the PDMS, a close peak between the PDMS and the PDMS + PEO-SA 2WT % was observed, due to the small concentration difference of PEO-SA present in its modification. 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.

The slightly and apparent increase of the C-C-O group in the IR spectra of the modified PDMS could indicate the presence of PEO-SA in the surface and very close layers (1.6µm) according to the ATR-FTIR method used. Despite the qualitative results obtained, this analysis and data does not confirm that the proposed reaction took place and the incorporation of PEO-SA into the PDMS was successful performed, so that, Nuclear Magnetic Resonance (NMR) Spectroscopy should be performed. This technique will allow to determine the content and molecular structure of the modified PDMS and corroborate the Hydrosilylation reaction proposed.

b) Water Contact Angle Analysis

The surface contact angle was measured to confirm a reduction of hydrophobicity in the surface of the PDMS. An 8µL drop of water was placed on the surface of the PDMS substrates and the contact angle of the interface was evaluated to compare surface hydrophobicity across polymers (Figure 25).

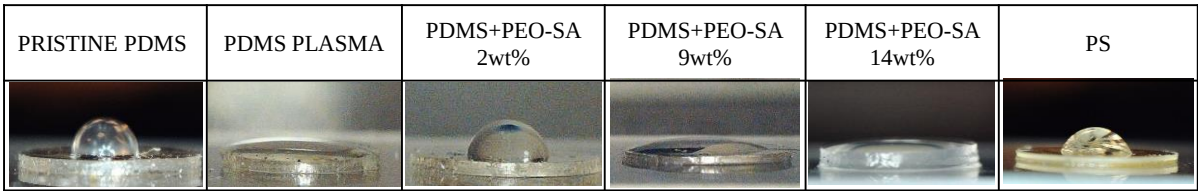

Figure 25. ***Water droplet contact angle on plane polymers surface.***

The water contact angle was evaluated in two different time intervals. The first interval was a short time monitored from 0-5 minutes (Figure 26.a). During the short time period the surface contact angle observed was 100º, 26º and 16º for PDMS + PEO-SA 2wt%, 9wt% and 14%respectively. The reduction in the contact angle was attributed to the migration of the PEO segments to the surface[7]. The PDMS PLASMA condition has the smallest contact angle below 15º. Both, the PRISTINE PDMS (~114°) and the PS (~85°) remain constant in their hydrophobic and hydrophilic properties, respectively[41].

In the Figure 26.b, the polymers were evaluated in a long-time interval of 0-3months (where the 0-time point corresponds to the measurement taken at 5min). As expected, PDMS PLASMA, had a contact angle similar to PRISTINE PDMS which is indicative of recovery of hydrophobic properties[21]. PDMS modified with PEO-SA retained contact angles observed at short time intervals indicating a permanent modification of the hydrophobic properties of the PDMS.

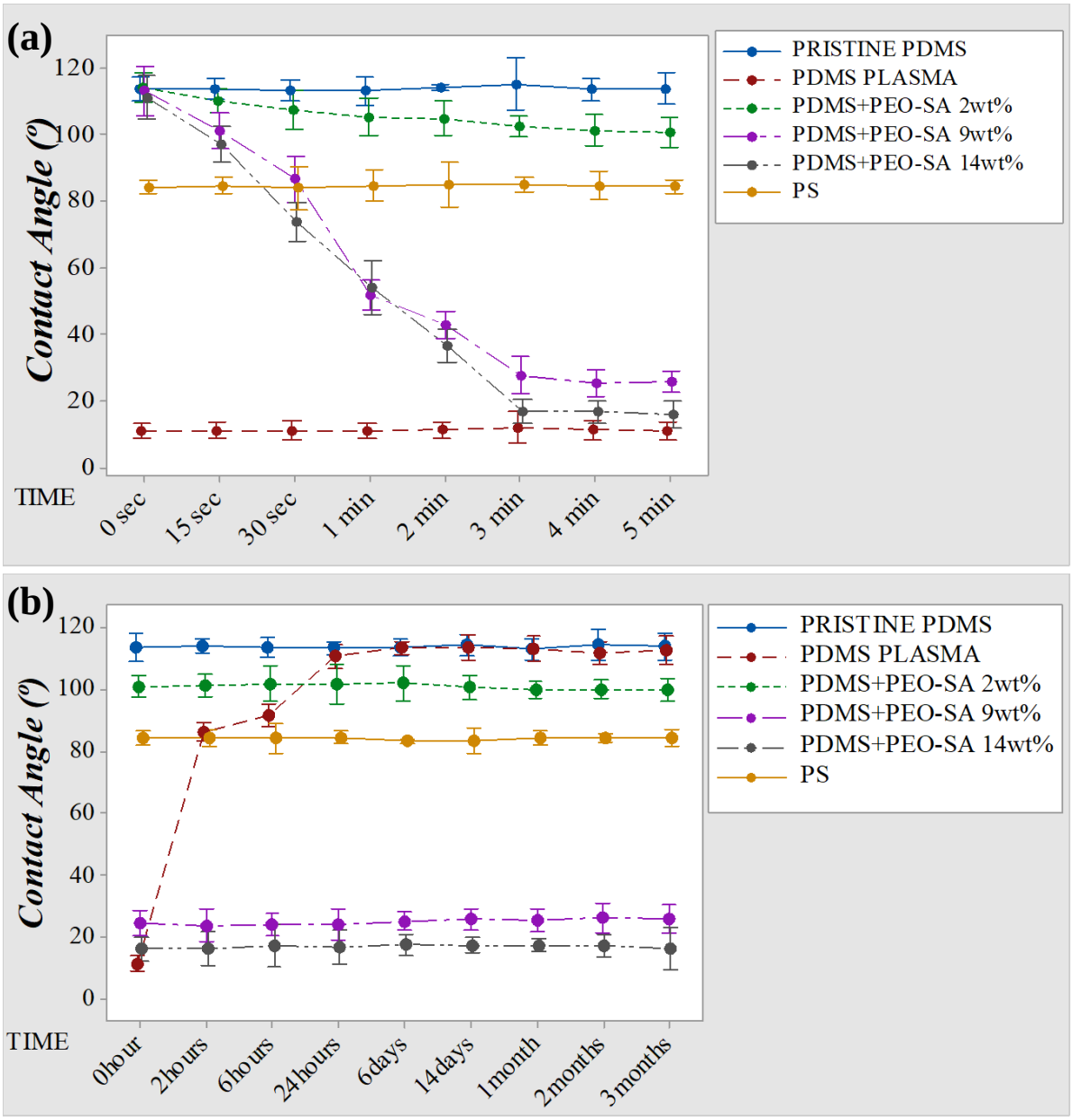

Figure 26. ***Static water contact angle measurements (°) of the polymers.*** *A volume 8µL water droplet on PRISTINE PDMS, PDMS PLASMA, PDMS+PEO-SA (2wt%, 9wt% and 14wt%) and PS. a) Measurements taken in an interval of 0 to 5 minutes. b) Measurements taken in an interval of 0 to 30 months. Each point represents the average of three water droplet measurements on three different single polymer discs at the same point.*

4.2 *Aim 2. To confirm optical transparency and reduction of hydrophobic absorption of small molecules into the PDMS-PEO-SA substrates*

a) Optical Transparency

Optical transparency is one of the characteristic properties of PDMS. This has allowed a wide range of applications in optical systems such as microfluidics devices and nanofabrication[69]. It was evaluated if the modification of the PDMS by addition of PEO-SA had an impact on the optical transparency of the PDMS substrates. Three polymer discs per condition were previously measured in thickness and were subsequently placed in the bottom of a 96-well plate and analyzed in UV / VIS Spectra Multiplate Reader (INFINITE 200 PRO, TECAN) at 600nm[43]. The absorbance values were divided by their corresponding thickness. The data was normalized and compared to PRISTINE PDMS.

Figure 27 shows the results obtained in absorbance. Although there were differences in the thickness of the PDMS substrates (PRISTINE PDMS= 0.48±0.3mm, 2wt%= 0.49±0.01mm, 9wt%=0.52±0.04mm and 14wt% =0.71±0.08mm), the results showed that there was no significant difference between them. This friendly test showed us that the incorporation of PEO-SA in PDMS has no modular impact on optical transparency.

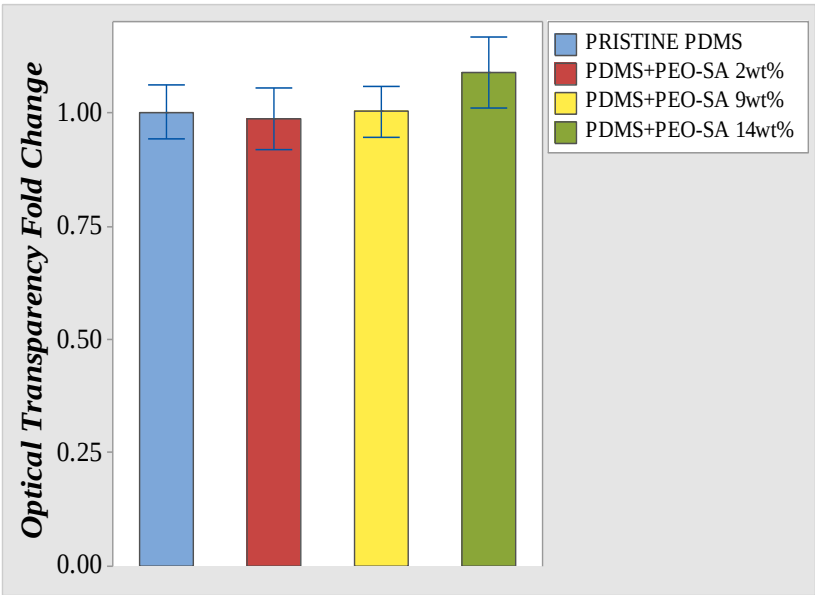

Figure 27. ***Optical transparency by UV/VIS absorbance.*** *The data was* ***normalized*** *with respect to the PRISTINE PDMS. Data represents average of 3 measurements +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05*

b) Hydrophobic/Hydrophilic Absorption

The geometry used in the absorption assays plays an important role in the diffusion of the small hydrophobic and hydrophilic molecules studied as drug models, since the contact area and the concentration are important factors in the absorption. Two different castings were made to the PDMS: discs and microwells. The results obtained, despite not being comparable given the difference in geometry and the type of analysis performed, allow us to corroborate that there was absorption of small molecules modulated by the incorporation of PEO-SA in the PDMS.

Nile Red and Trypan Blue were selected for their molecular size and fluorescent/ colorimetric property to optically monitor absorption into the PDMS substrates.

Nile Red is a lipophilic fluorophore with high selectivity and sensitivity. When a hydrophobic-rich environment is present the fluorescence intensity is strong. The PDMS to has siloxane in it composition, mimic this environment[14]. This behavior of the Nile Red, allowed us to quantitatively evaluate the hydrophobic absorption into the modified PDMS. A microwell (~2mm diameter) of each polymer was placed on a microscopy slide, and 3µL of 1mM Nile Red was added. Texas Red fluorescence filter was set in a Fluorescence Microscope (PRO282B, MOTIC) and the images were taken at 4x magnification.

In the figure 28, representative fluorescence images of Nile Red absorbed into the PDMS substrates at 15 seconds, 5 minutes and 30 minutes were taken. It was observed that the PRISTINE PDMS in contact with the droplet of Nile Red emitted a greater fluorescence compared to the rest of the polymers, this because the hydrophobic molecule of Nile Red is absorbed within the bulk PDMS. When analyzing the PDMS + PEO-SA 2wt% and 9wt% fluorescence images, a hydrophobic behavior can be observed 15 seconds after the test, because the surface gradually undergoes a reorganization in its segments to reach hydrophilicity at 3 minutes, as observed in the water contact angle assay. PDMS + PEO-SA 9wt% and 14wt% present an absorption similar to PS[14], and a stable hydrophilic behavior over 30 seconds (Figures 35-37 Appendix).

In these fluorescence images a reduction in Nile Red absorption was observed, modulated by the increase in concentration of PEO-SA within the PDMS.

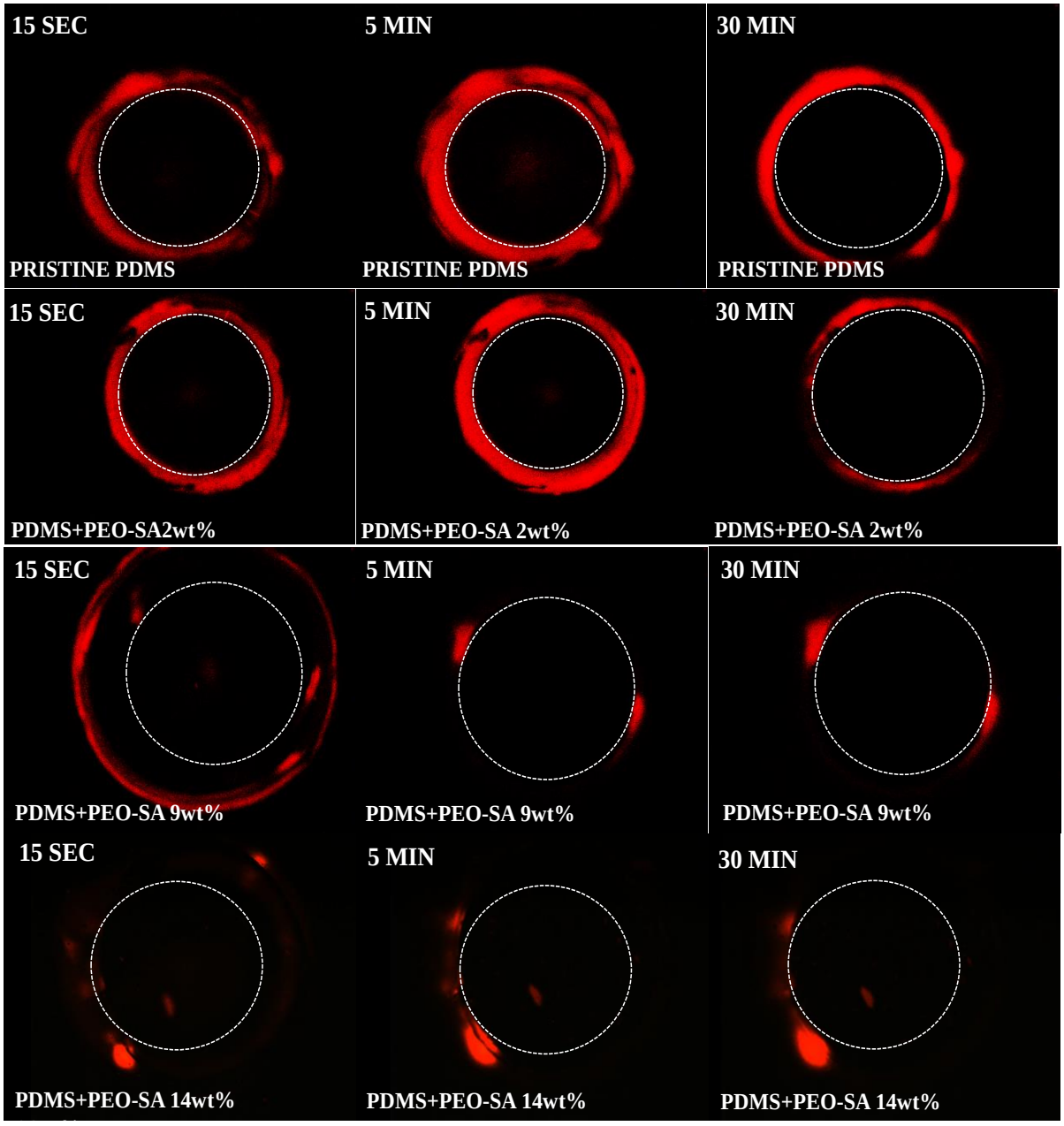

Figure 28. ***Representative fluorescence images of Nile Red absorbed into polymers***. *Images were taken at 15seconds, 5 minutes and 30 minutes to capture the absorption of Nile Red into a PRISTINE PDMS, PDMS+PEO-SA 2wt%, PDMS+PEO-SA 9wt% and PDMS+PEO-SA 14wt% microwells. 3uL droplet of Nile Red (1mM stock) was pipetted inside to each microwell. The inside area of the microwell is marked with dashed-line white circle of a diameter of 2mm.*

The images at 30 minutes were later analyzed with the ImageJ v.1.50i software (NIH, MD-USA) and the surface fluorescence intensity of the PDMS substrates was measured. The results are shown in the following table:

Table 6. ***Fluorescence Surface Area of Nile Red absorption into the PDMS substrates.***

POLYMER	***AREA ($x10^3$ pixels^2)***
PRISTINE PDMS	33.6767
PDMS+PEO-SA 2wt%	24.0815
PDMS+PEO-SA 9wt%	10.3490
PDMS+PEO-SA 14wt%	4.8400

The results of the measurement of fluorescence surface area, showed a decrease in the absorption of Nile Red, and consequently a reduction in hydrophobicity of 28% for the PDMS + PEO-SA 2wt%, 69% for the PDMS + PEO-SA 9wt% and 85% for the PDMS + PEO-SA 14wt% compared with the PDMS. An unequal distribution in Nile Red absorption into the modified PDMS, may indicate that there is no homogeneity in the preparation of PDMS + PEO-SA.

Trypan Blue was selected as a model hydrophilic drug and a possible increase in the absorption of hydrophilic molecules by addition of PEO-SA into PDMS was evaluated. A microwell (~2mm diameter) of each polymer was placed on a microscopy slide, and 3µL of 1mM Trypan Blue was added. Brightfield filter was set in a Fluorescence Microscope (PRO282B, MOTIC) and the images were taken at 4x magnification.

In the Figure 29 representative brightfield images of Trypan Blue in the microwells at 0 seconds, 20 minutes and 40 minutes were taken. The measurement at time 0 allowed us to visualize the well before adding the dye, and thus be able to make the comparisons against a possible absorption. It was observed that PRISTINE PDMS and PDMS + PEO-SA 2wt% did not sequester the trypan blue molecule in its interior, unlike PDMS + PEO-SA 9wt% and 14wt% showed an absorption of the dye at 40 minutes of the test. This was attributed to the demonstrated improvement in hydrophobicity that polymers have when the concentration of PEO-SA is increased.

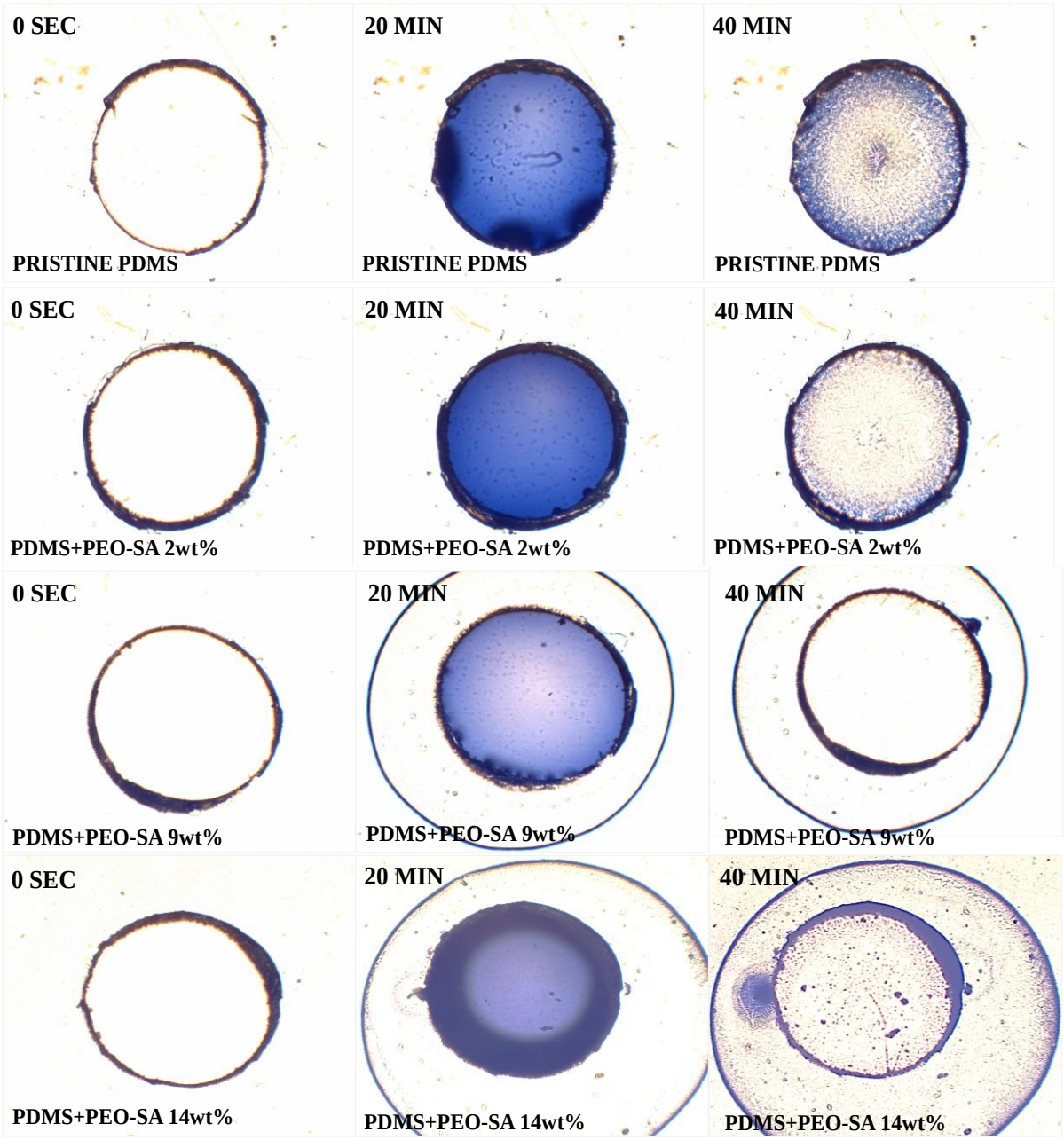

Figure 29. ***Representative brightfield images of Trypan Blue absorbed into polymers.*** *Images were taken at 0s, 20 minutes and 40 minutes to capture the absorption of Nile Red into a PRISTINE PDMS, PDMS+PEO-SA 2wt%, PDMS+PEO-SA 9wt% and PDMS+PEO-SA 14wt% microwells. 3uL droplet of Trypan Blue (1mM stock) was pipetted inside to each microwell. The inside area of the microwell have a diameter of 2mm.*

The brightfield at 40 minutes were later analyzed with the ImageJ v.1.50i software (NIH, MD-USA) and the surface absorption area of the polymers was measured. The results are shown in the following table:

Table 7. ***Brightfield Surface Area of Trypan Blue absorption into the PDMS substrates.***

POLYMER	***AREA (mm)^2***
PRISTINE PDMS	0
PDMS+PEO-SA 2wt%	0
PDMS+PEO-SA 9wt%	74.4730
PDMS+PEO-SA 14wt%	125.8423

The reduction in hydrophobicity by the addition of PEO-SA produced a slight unexpected impact on the absorption of hydrophilic molecules. For this reason, the absorption of the hydrophilic drug Doxorubicin Hydrochloride was evaluated.

The images showed a non-homogenous Nile Red and Trypan Blue absorption into the PDMS + PEO-SA 9wt% and 14%, therefore a presumable non-homogeneous incorporation of PEO-SA into the bulk PDMS during the mixing of the oligomers before curing was carried out.

4.3 ***Aim 3. To determine biocompatibility of the PDMS substrates***

a) Viability Assay

The metabolic cell activity was measured by XTT absorbance at 465nm using UV/VIS Spectra Multiplate Reader (INFINITE 200 PRO, TECAN).

MCF-7, MDA-MB-231, MDA-MB-468 and NIH-3T3 cytotoxicity influenced by the PDMS substrates was evaluated (Figure 30). The data was normalized to TCP (no polymer floating) as viability control. In the MCF-7 cells (Figure 30.a), the viability values of PRISTINE PDMS, PDMS+PEO-SA 2wt%, 9wt% and 14wt% were similar to TCP (~100%). No expected increase in viability was observed in cells exposed to PDMS + PEO-SA 2wt%, 9wt% and 14wt%, with respect to PRISTINE PDMS since previous studies showed that PDMS

is capable of sequestering hydrophobic molecules such as the estrogen[47] present in the phenol network of the culture medium.

When MDA-MB-231, MDA-MB-468 and NIH-3T3 cell viability was evaluated, showed that there was a significant difference in viability of cells exposed to PDMS+PEO-SA 9wt% and 14% (Figure 30.b), PDMS+PEO-SA 14% (Figure 30.c) and PDMS+PEO-SA 9wt%, 14% (Figure 30.d) respectively registered values over 80%. Conversely, PRISTINE PDMS and PDMS+PEO-SA 2wt% registered a similar viability to TCP.

Despite this, the PDMS 9wt% and 14% were not considered to be cytotoxic since values above 80% are considered non-toxic[70,71].

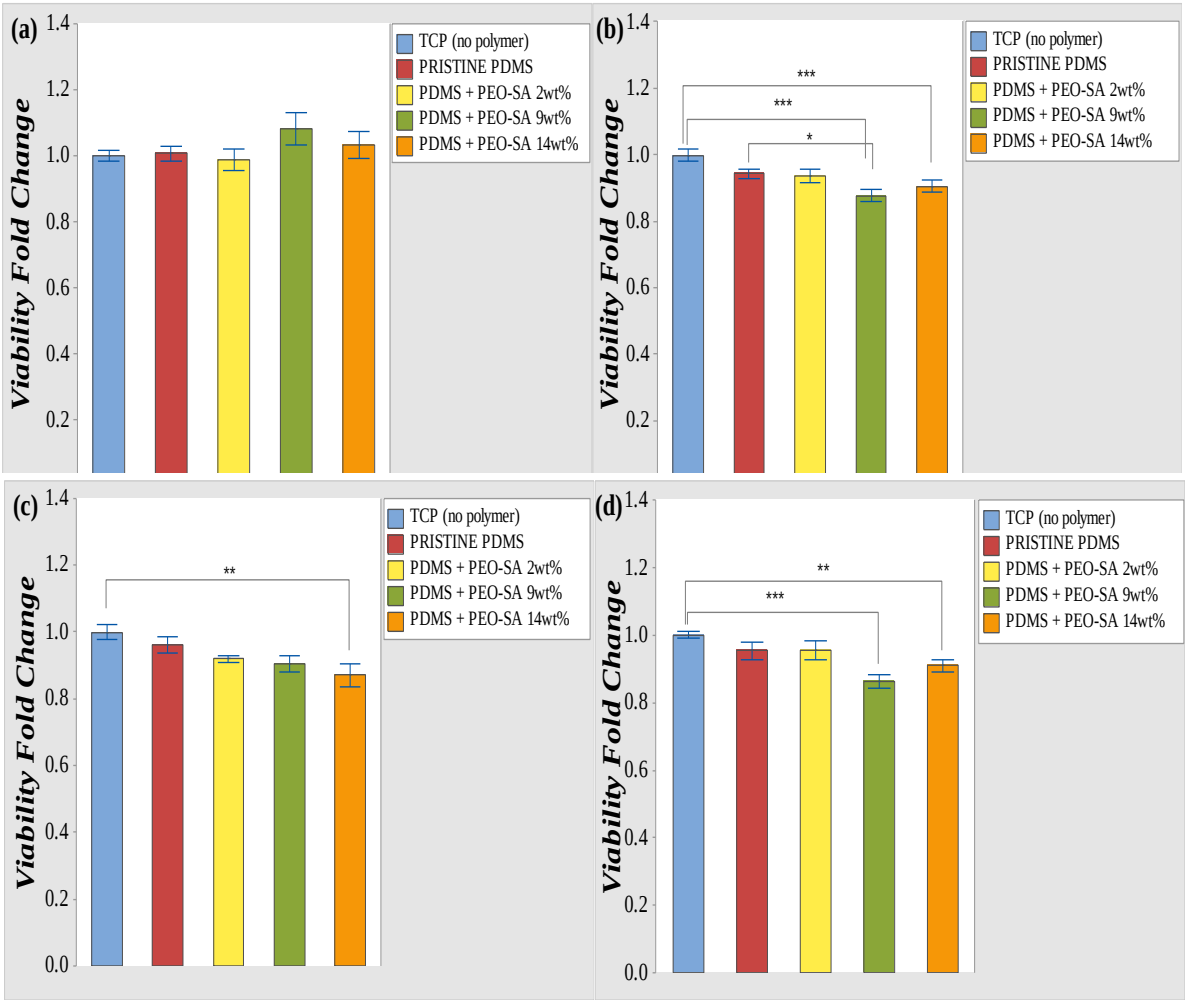

Figure 30. ***Biocompatibility of the PDMS substrates by Viability assay.*** *Cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Viability experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to the TCP.* ***(a)*** *Viability of* ***MCF-7*** *cells.* ***(b)*** *Viability* ***of MDA-MB-231*** *cells.* ***(c)*** *Viability of* ***MDA-MB-468*** *cells.* ***(d)*** *Viability of* ***NIH-3T3****. Data represents average of 5,3, 3 and 4 independent experiments with n=6,6,4 and 6 wells +/-1 SE, respectively. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*

In the Figure 38 Appendix, cell line morphologies were evaluated and compared with cells seeded in TCP (no polymer floating). In the post-treatment (incubation for 48h) of the cells exposed to the PDMS substrates, no significative changes were found in cell morphology as expected, since the incorporation of PEO-SA into the PDMS did not have a greatly modulating effect on the biocompatibility[7,8,13].

The polymeric discs were submerged in the culture medium, without having direct contact with the cell lines seeded in the bottom of each well, to evaluate biocompatibility. The cells were not placed in microwells due to the decrease in the adhesion on TCP of the PDMS+PEO-SA 9wt% and 14wt%. Nevertheless, the viability of the MDA-MB-231 cells seeded in TCP was evaluated, being surrounded by the microwell of PRISTINE PDMS and PDMS+PEO-SA 2wt% showing results over 80% as was demonstrated in this assay using single polymer discs (Figure 39 Appendix).

b) Cell Proliferation Assay

Cell proliferation is a fundamental assay to determine genotoxicity by measuring of DNA synthesis, based in a click reaction, and detecting the incorporation of EdU with the Alexa Fluor 594 without denaturing the DNA[49,57].Proliferation in MCF-7, MDA-MB-231, MDA-MB-468 and NIH-3T3 cell lines exposed for 48 hours to PRISTINE PDMS, PDMS+PEO-SA 2wt%, 9wt% and 14wt% discs were evaluated, and compared with cells seeded in TCP (no polymer floating).

Figure 31 shows results obtained in the proliferation test. Similar proliferation results respect to TCP were found in PRISTINE PDMS and PDMS+PEO-SA 2wt%. PEO-SA (2wt%) did not have a significant impact on the cellular response.

Proliferation rates fell below 50% in PDMS+PEO-SA 9wt% and 14wt% for the MCF-7 cell line (Figure 31.a). The same effect was observed in MDA-MB-231 (Figure 31.b), MDA-MB-468 (Figure 31.c) and NIH-3T3 (Figure 31.d). Results indicate that a negative effect in the proliferation rates of all cell lines at PEO-SA concentrations above 9%. This effect may be caused by absorption of vital hydrophilic molecules presents in the culture medium as

prolactin[47,56], or by the leaching of non-crosslinked PEO-SA that was not removed in the oligomeric extraction, where ethane was used, a non-polar solution, with the ability to leach the hydrophilic oligomers of the modified PDMS, which may have had some interaction with the cells or with the components present in the culture medium[3]. Or maybe a no expected reduction in the oxygen permeability modulated by the addition of PEO-SA into the PDMS no measured in this work.

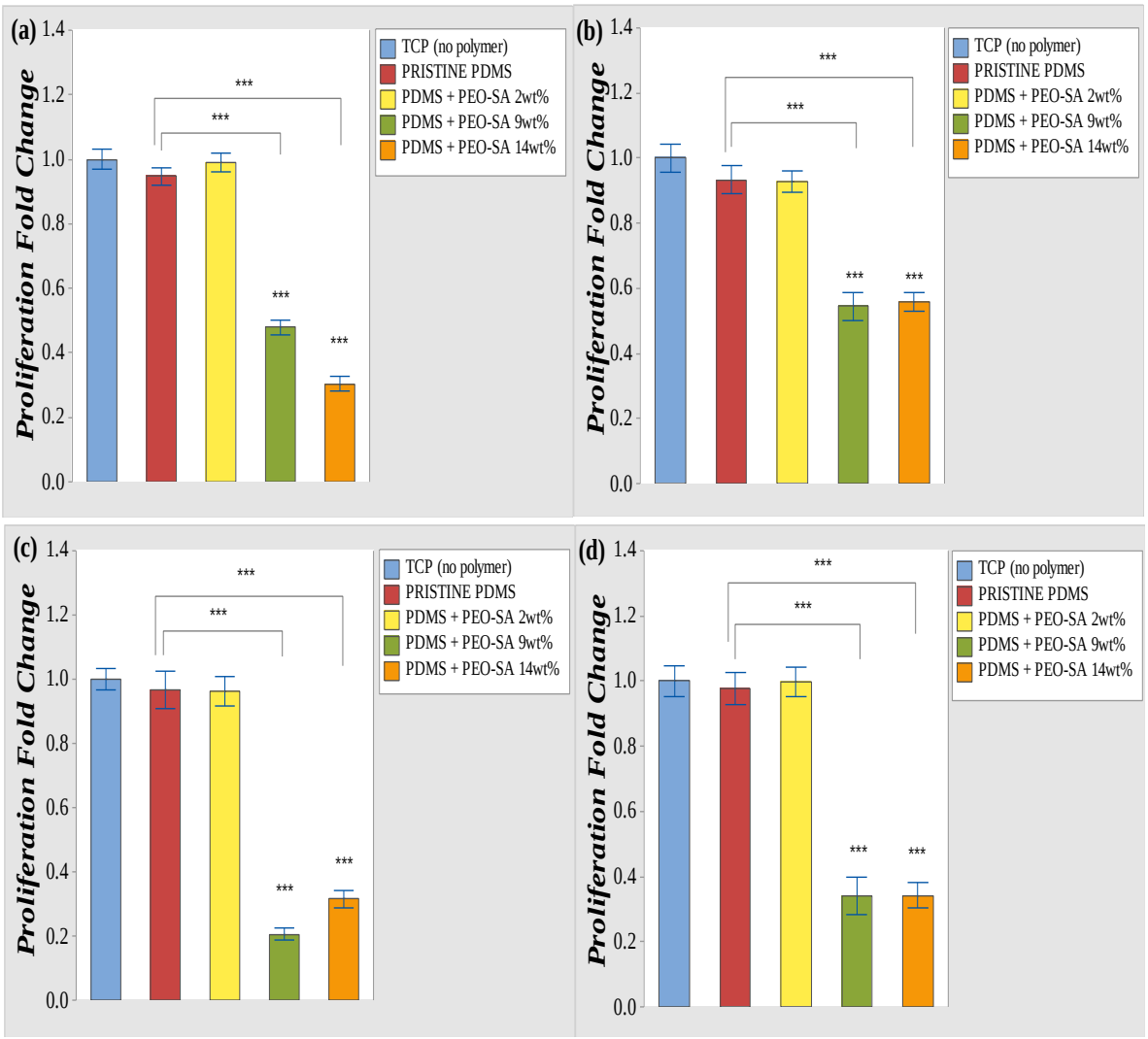

Figure 31. ***Biocompatibility of PDMS substrates by Proliferation assay.*** *Cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Proliferation experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to the TCP.* ***(a)*** *Proliferation of* ***MCF-7*** *cells.* ***(b)*** *Proliferation* ***of MDA-MB-231*** *cells.* ***(c)*** *Proliferation of* ***MDA-MB-468*** *cells.* ***(d)*** *Proliferation of* ***NIH-3T3****. Data represents average of 3 independent experiments with n=5 wells +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*

4.4 *Aim 4. To confirm that cell response to hydrophobic molecules is improved in PDMS+PEO-SA substrates*

Absorption impact in the cellular response

The efficacy in absorption of small hydrophobic/hydrophilic molecules into the PDMS substrates was evaluated by the inversely proportional relationship among the hydrophobic/hydrophilic absorption and the viability. The absorption impact in the cellular response was evaluated in MCF-7, MDA-MB-231 and MDA-MB 468 cell lines.

In the Figure 32.a, the impact of hydrophilic drug DOX.HCL absorption on the viability of MCF-7 cells was evaluated. Cells were treated +/- DOX.HCL and normalized to TCP (no polymer floating). A reduction of ~ 30% in viability was observed in TCP (no polymer floating), PRISTINE PDMS, PDMS + PEO-SA 2wt%, 9wt% and 14wt%, there was no strong difference in viability results (Figure 32.b), showing that that no changes in cell behavior suggesting a minimal hydrophilic absorption of DOX.HCl.

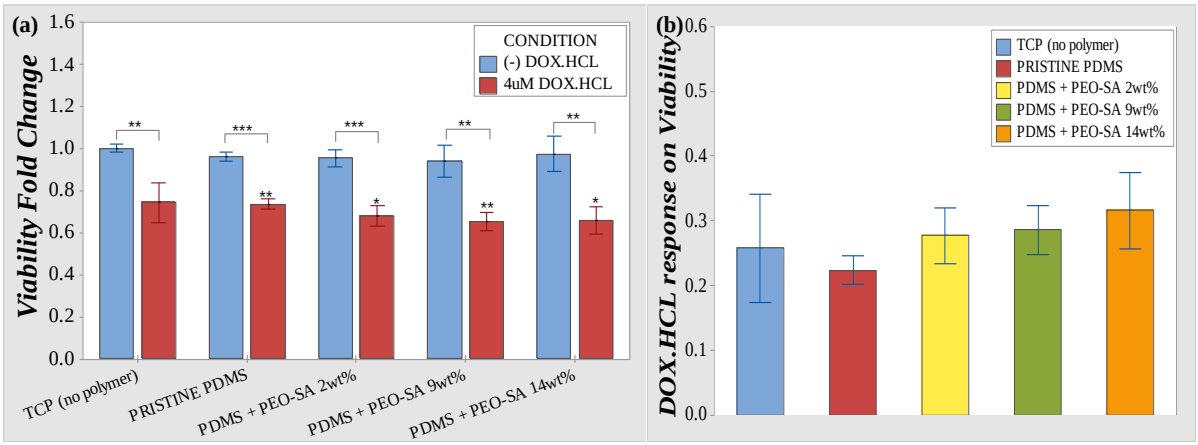

Figure 32. ***Viability of MCF-7 cells in response to DOX.HCL.*** *MCF-7 cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Wells were dosed **+/-4µM DOX.HCL** and then single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Viability experiments were performed **48h post treatment**. **(a)** Viability of MCF7 cells **+/-4µM DOX.HCL**. **(b)** Data represents difference between **+/-4µM DOX.HCL** from Figure 32.a. The data was **normalized** with respect to the TCP. Data represents average of 3 independent experiments with n=4 wells +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*

MDA-MB-231 and MDA-MB-468 cells viability and response to hydrophilic absorption of DOX.HCL was also evaluated. The Figures 33.a,c show the results obtained when the cells were treated +/- DOX.HCL in the presence of the PDMS substrates and normalized to TCP (no polymer floating) A reduction of ~ 25% in viability of MDA-MB-231

cells was observed in TCP (no polymer floating), PRISTINE PDMS and PDMS + PEO-SA 2wt%. Respect the PDMS + PEO-SA 9wt% and 14wt%, when were compared with the control (TCP) a reduction in the drug impact of ~3% and ~7% respectively, was observed. (Figure 33.b). A similar behavior in MDA-MB-468 cells was observed. In Figure 33.c, a reduction of drug impact and response on viability in PDMS + PEO-SA 9wt% of 7%, compared with the TCP, was observed. These results showed that there was an impact on viability modulated by the incorporation of PEO-SA 9wt% and 14wt% suggesting a hydrophilic absorption of DOX.HCl similar to the results of Trypan Blue absorption, despite being analizyed using a different polymeric geometry.

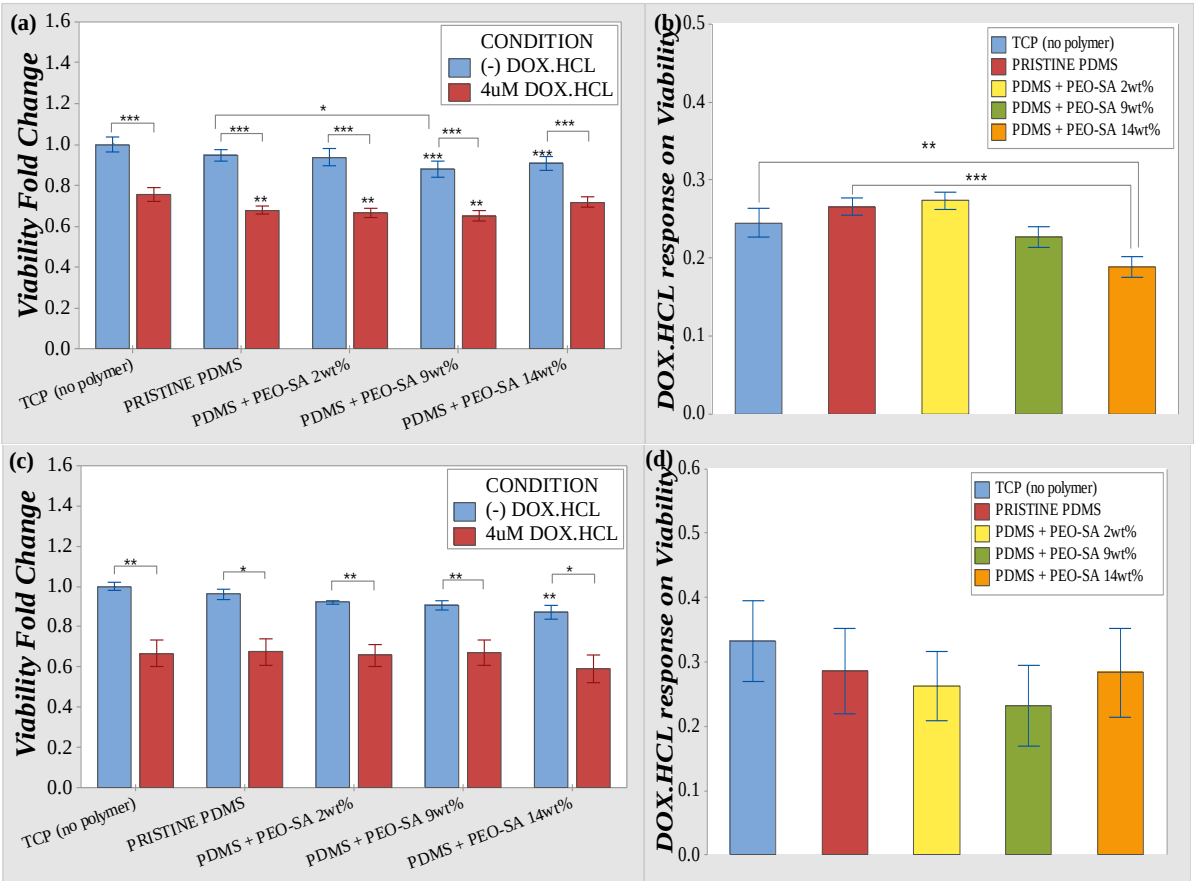

Figure 33. ***Viability of MDA-MB-231and MDA-MB-468 cells in response to DOX.HCL.*** *Cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Wells were dosed* ***+/-4µM DOX.HCL*** *and then single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Viability experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to the TCP.* ***(a)*** *Viability of MDA-MB-231 cells* ***+/-4µM DOX.HCL****.* ***(b)*** *Data represents difference between* ***+/-4µM DOX.HCL f****rom Figure 33.a.* ***(c)*** *Viability of MDA-MB-468 cells* ***+/-4µM DOX.HCL****.* ***(d)*** *Data represents difference between* ***+/-4µM DOX.HCL f****rom Figure 33.c. Data represents average of 4 independent experiments with n=6 wells +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*

The estrogen-dependent MCF-7 cell line was used for the study of β-Estradiol and DOX.HCL response on viability. In the Figure 34.a viability results are shown +/- treatment with 0.1nM β-Estradiol normalized to TCP (no polymer floating). As the MCF-7 cells are estrogen-dependent, an impact on the reduction of viability when the cells were in contact with the PDMS substrates was expected, since previous studies showed that estrogen is sequestered into the PDMS[3].

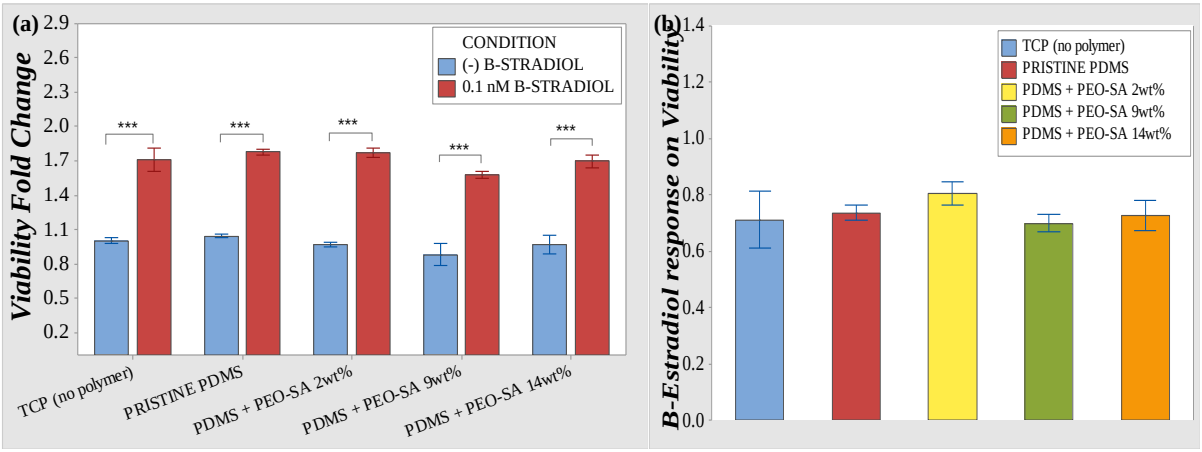

Figure 34. ***Viability of MCF-7 cells in response to β -Estradiol.*** *MCF-7 cells were cultured in DMEM+10%FBS charcoal stripped serum for 72h. Wells were dosed* ***+/-0.1nM β-Estradiol*** *and then single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Viability experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to the TCP.* ***(a)*** *Viability of MCF-7 cells* ***+/-0.1nM β-Estradiol****.* ***(b)*** *Data represents difference between* ***+/-0.1nM β-Estradiol*** *from Figure 34.a. Data represents average of 3 independent experiments with n=4 wells +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*

The results showed that there was an increase of ~ 70% in viability in TCP (no polymer floating), PRISTINE PDMS, PDMS + PEO-SA 2wt%, 9wt% and 14wt% when they were compared with MCF-7 cells without treatment. Viability in both, estrogen-treated and untreated cells did not show a significant difference or impact on viability (Figure 34.b), thus demonstrating that absorption of β-Estradiol there was no impact in cell growth at the concentration of 0.1nM, as expected[3]. This could be since the used concentration of β -Estradiol exceeded the amount required by the cells for their duplication, not reflecting an impact of absorption on viability. This was corroborated in the evaluation of the proliferation obtaining the same results, there was no impact on the proliferation by absorption of the polymers. (Figure 40 Appendix). To show that modified PDMS improves sensitivity of cell-based assays with hydrophobic molecules as compared to PDMS, should be used the MCF-7 MVLN luciferase reporter cell line for estrogen receptor signaling –increases the sensitivity of our assay to perceive changes in estrogen concentration[3].

CHAPTER 5 – CONCLUSION

Based on the objectives presented in this work, we present the most important conclusions finding in our investigation.

5.1 The incorporation of PEO-SA into the PDMS was confirmed via FTIR and measurement of the contact angle on the surface of the PDMS substrates.

The group C-C-O, characteristic group of compounds with PEO, was slightly observed at the 915-865 cm^{-1} wavenumber in the modified PDMS. Nuclear Magnetic Resonance (NMR) Spectroscopy should be performed to determine the content and molecular structure of the modified PDMS and corroborate the Hydrosilylation reaction proposed.

The measurements of the contact angle show that the surface hydrophobicity of the PDMS was reduced proportionally to the concentration of PEO-SA added into the PDMS. Contact angles in PDMS modified with PEO-SA were maintained for a period of up to three months.

5.2 Optical transparency was evaluated using UV / VIS absorbance measured at 600nm wavelength. Comparisons across PDMS substrates confirmed no significant difference compared to pristine PDMS. Small molecule absorption was qualitatively and indirectly evaluated by fluorescent microscopy and cell viability analysis respectively. Results with Nile Red showed that there is a significant reduction in absorption of this hydrophobic molecule proportional to the concentration of PEO-SA. Results with Trypan Blue confirmed absorption of this hydrophilic molecule in PDMS + PEO-SA 9wt% and 14wt% but not 2wt%. An unequal distribution in Nile Red absorption into the modified PDMS, may indicate that there is no homogeneity in the preparation of PDMS + PEO-SA.

5.3 Metabolic cell activity was measured by XTT absorbance at 465nm using UV/VIS Spectra Multiplate Reader. Biocompatibility of the PDMS substrates was corroborated by cell viability levels above 80% in a several cell lines. Results of cell proliferation rates show rates below 50% for PEO-SA concentrations at or above 9wt%. PEO-SA (2wt%) did not have a significant impact on cell growth.

5.4 Cellular assays using DOX.HCL (hydrophilic drug) show for PEO-SA concentrations at or above 9wt% changes in cell behavior, suggesting that there was a hydrophilic absorption in the substrates of PDMS. Cell viability assays in the presence of β-Estradiol (model hydrophobic drug) show cell viability levels in pristine PDMS and PEO-SA modified PDMS similar to TCP. The concentration of β-Estradiol used although at physiological levels may have been too high to such an extent that changes in estradiol concentration were not perceived at the cellular levels. To show that PDMS + 2% PEO-SA improves sensitivity of cell-based assays with hydrophobic molecules as compared to PDMS, should be used the MCF-7 MVLN luciferase reporter cell line for estrogen receptor signaling –increases the sensitivity of our assay to perceive changes in estrogen concentration.

The current work established that the PDMS modified by addition of PEO-SA at 2wt% is a potential option for replacing the pristine PDMS for cellular assays in which small hydrophobic molecules are involved in cellular response and micro and nano fabrication of fluidics devices.

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Author: M. A. Rufin,J. A. Gruetzner,M. J. Hurley,M. L. Hawkins,E. S. Raymond,J. E. Raymond,M. A. Grunlan

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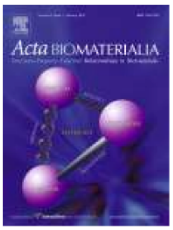

Title:	Protein resistance efficacy of PEO-silane amphiphiles: Dependence on PEO-segment length and concentration
Author:	Marc A. Rufin,Mikayla E. Barry,Paige A. Adair,Melissa L. Hawkins,Jeffery E. Raymond,Melissa A. Grunlan
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APPENDIX B – FIGURES

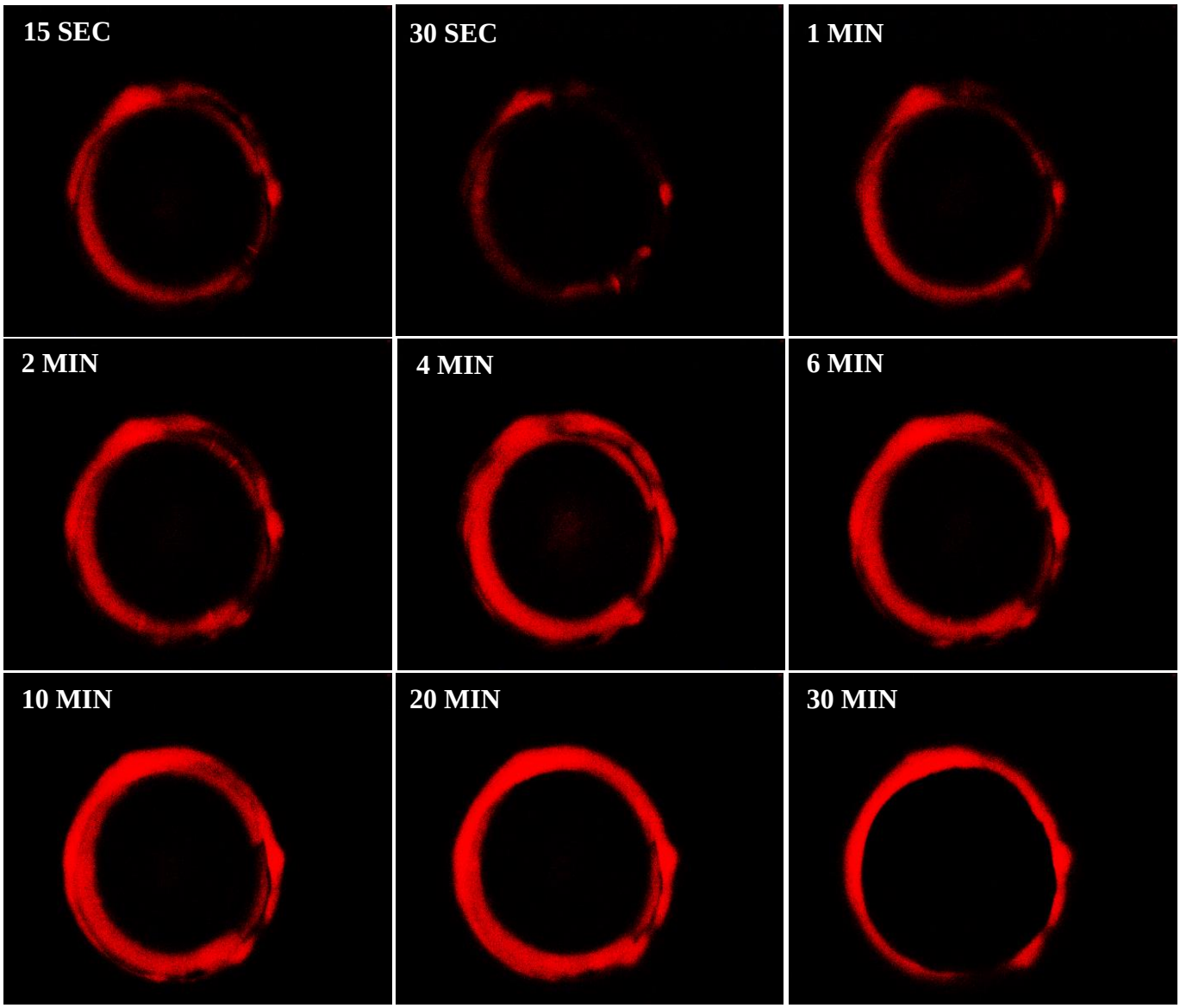

Figure 35. ***Representative fluorescence images of Nile red absorbed into PRISTINE PDMS****. Images were taken at 15-30 min to capture the absorption of Nile Red into a* ***PRISTINE PDMS*** *microwell. 3uL droplet of Nile red (1mM stock) was pipetted inside a microwell. The inside area of the microwell is marked with dashed-line white circle of a diameter of 2mm.*

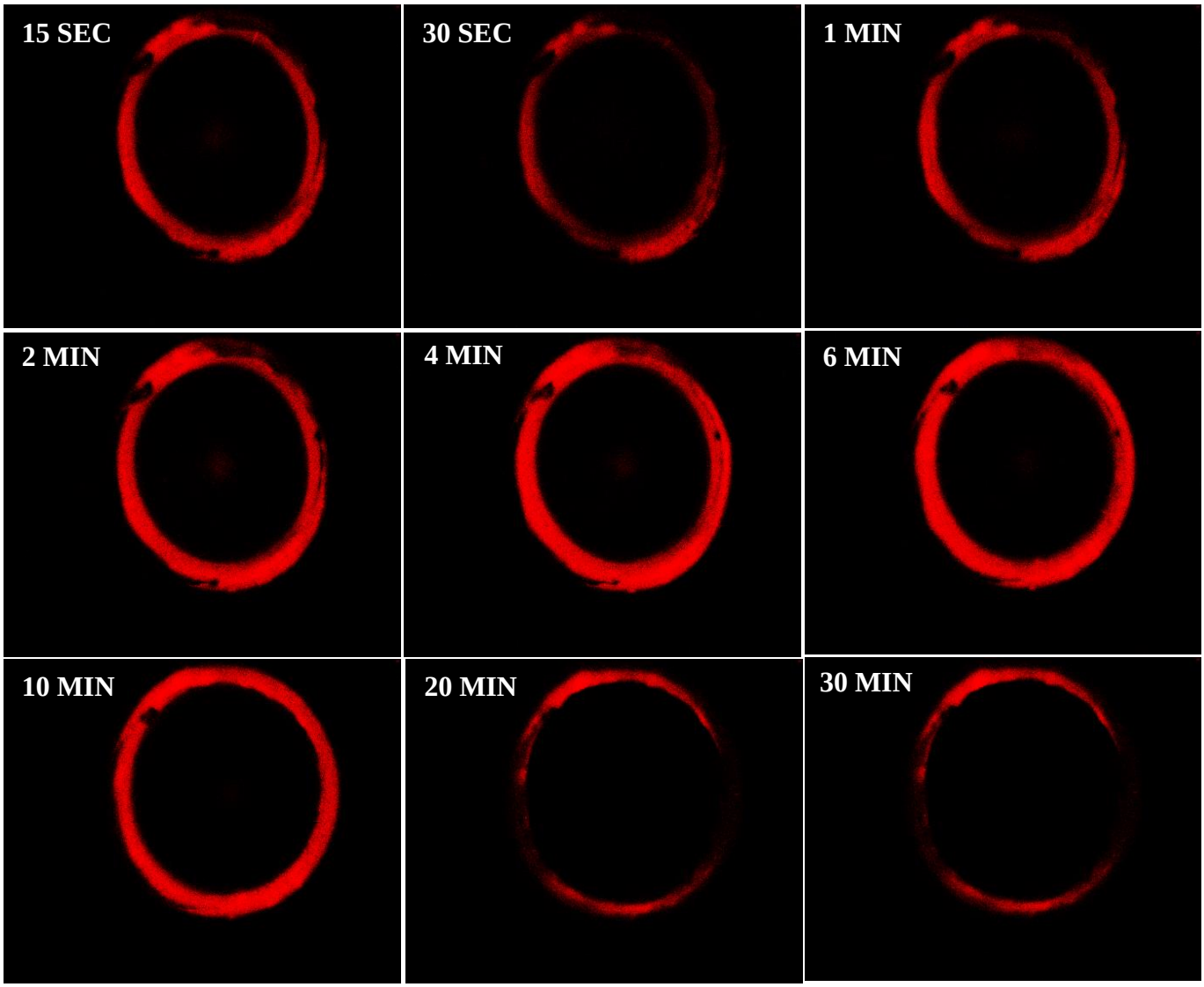

Figure 36. ***Representative fluorescence images of Nile red absorbed into PDMS+PEO-SA 2wt%****. Images were taken at 15-30 min to capture the absorption of Nile Red into a* ***PDMS+PEO-SA 2wt%*** *microwell. 3uL droplet of Nile red (1mM stock) was pipetted inside a microwell. The inside area of the microwell is marked with dashed-line white circle of a diameter of 2mm.*

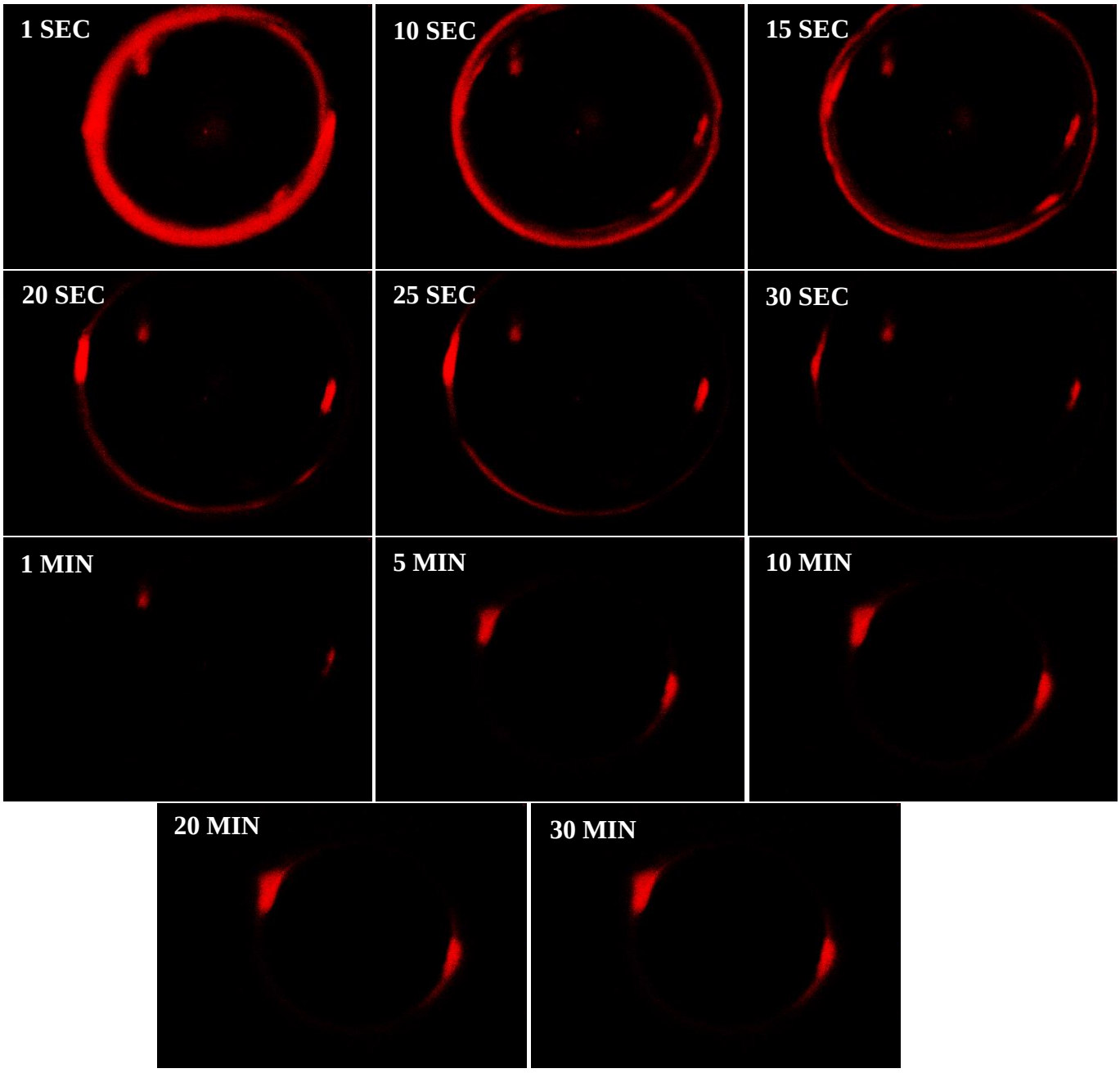

Figure 37. ***Representative fluorescence images of Nile red absorbed into PDMS+PEO-SA 9wt%****. Images were taken at 15-30 min to capture the absorption of Nile Red into a* ***PDMS+PEO-SA 9wt%*** *microwell. 3uL droplet of Nile red (1mM stock) was pipetted inside a microwell. The inside area of the microwell is marked with dashed-line white circle of a diameter of 2mm.*

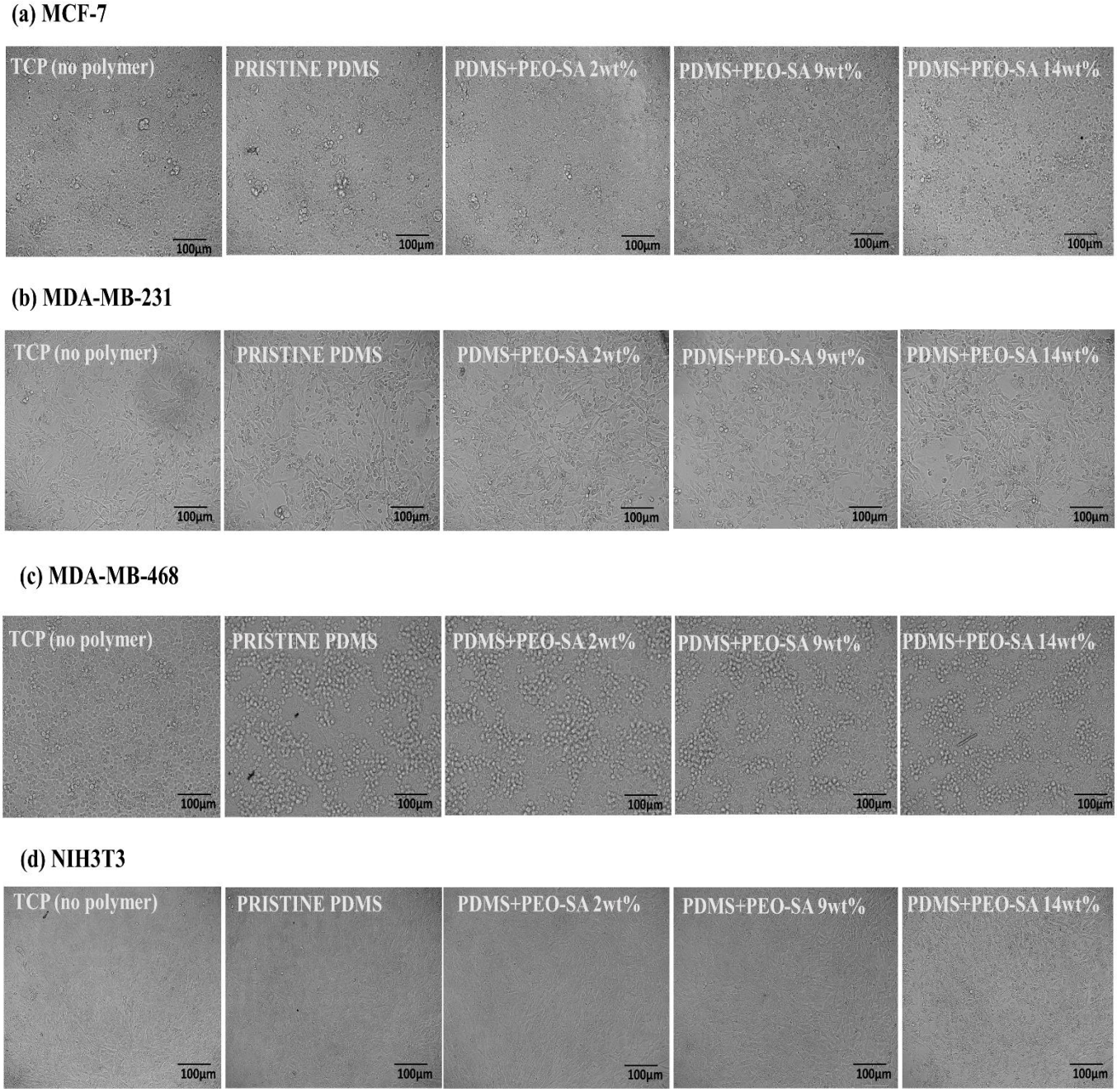

Figure 38. ***Brightfield images of cell morphologies exposed to single polymer discs***. *MCF-7, MDA-MB-231, MDA-MB-468 and NIH-3T3 cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Images were taken* ***48h post treatment****.*

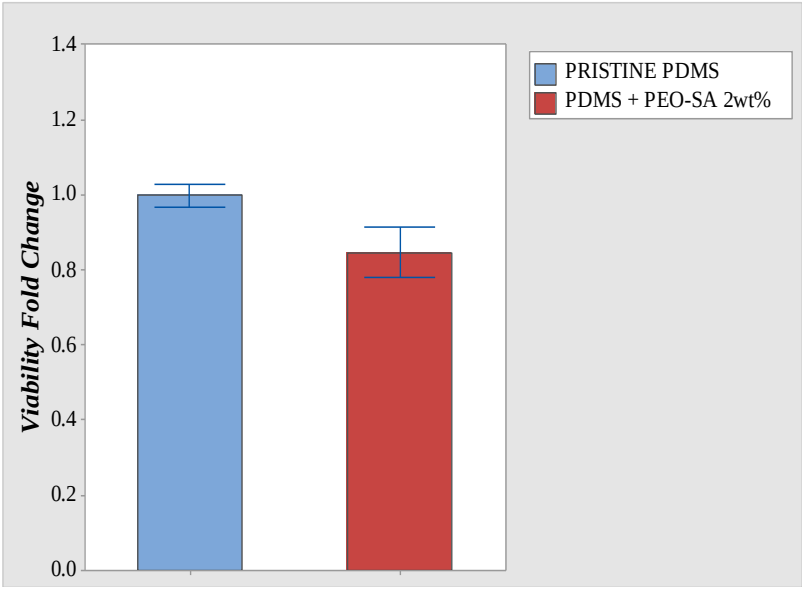

Figure 39. ***Biocompatibility of PDMS and PDMS+PEOSA 2wt% microwells by Viability assay in MDA-MB-231.*** *Cells were cultured in DMEM+10%FBS+1%P/S serum for 72h. Cells were seeded in PDMS and PDMS+PEO-SA 2wt% microwells. Viability experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to PDMS. Data represents average of 2 independent experiments with n=3 wells +/- 1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05*

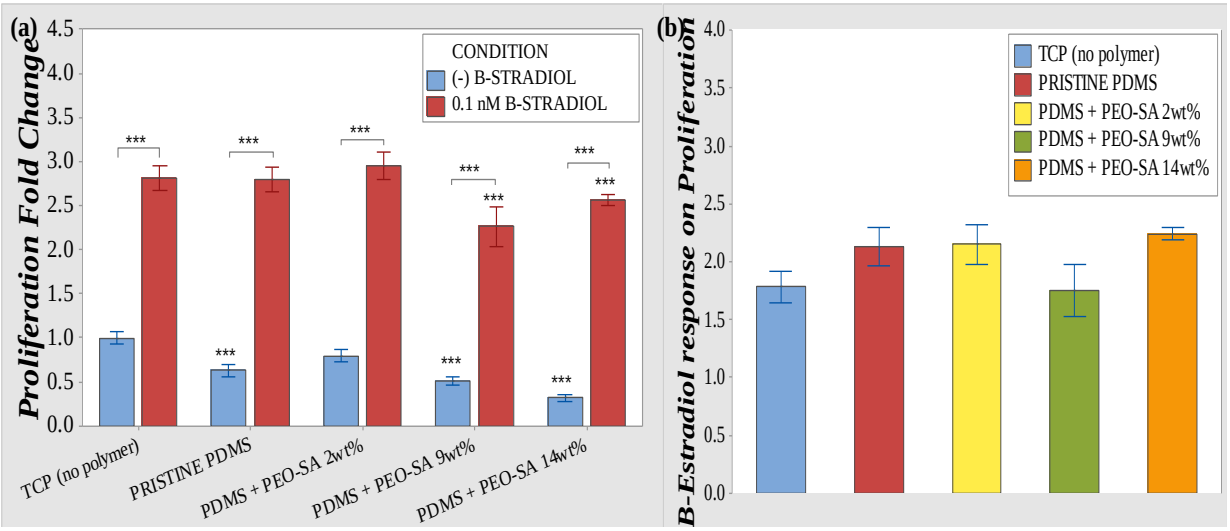

Figure 40. ***Proliferation of MCF-7 cells in response to β-Estradiol.*** *MCF-7 cells were cultured in DMEM+10%FBS charcoal stripped serum for 72h. Wells were dosed* ***+/-0.1nM β-Estradiol*** *and then single polymer discs were added to experimental 96-well plate. TCP had no single polymer discs. Viability experiments were performed* ***48h post treatment****. The data was* ***normalized*** *with respect to the TCP.* ***(a)*** *Viability of MCF-7 cells* ***+/-0.1nM β-Estradiol****.* ***(b)*** *Data represents difference between* ***+/-0.1nM β-Estradiol*** *from Figure 40.a. Data represents average of 3 independent experiments with n=5 wells +/-1 SE. Statistical analysis was done using single-factor analysis of variance (ANOVA), ns P>0.05, *P<0.05, **P<0.01, ***P<0.001*