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Assessment of Normal Human Breast Cells (MCF10A Cells)–Surface Interactions on Porous Poly-Di-Methyl-Siloxane (PDMS) Structures for Potential Biomedical Applications

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Abstract

Biocompatibility stands out as a crucial and fundamental requirement before approval of biomaterials for medical use. The study aimed to evaluate the interaction between normal human breast cells (MCF10A cells) and porous poly-di-methyl-siloxane (PDMS) structures for potential biomedical applications. Preparation and characterization of the PDMS substrate were carried out, followed by the assessment of cell proliferation and fluorescence imaging using an Alamar blue assay and fluorescence microscopy, respectively. The results revealed that initially (at 4 hours post-incubation), there was no notable difference in cell proliferation among the various groups (non-porous PDMS, PDMS_0-150, PDMS_150-250, and PDMS_250500). However, at 48 and 96 hours, a significant increase in cell proliferation was observed in the PDMS_250–500 µm group compared to other groups ($P<0.05$). Furthermore, the results of the fluorescence microscopy corroborated a substantial enhancement in cell growth and attachment as the porosity of the PDMS substrate increased. However, cells seeded on non-porous PDMS surfaces exhibited a significant decline ($P<0.05$) in cell growth

in both the Alamar blue assay and fluorescence imaging. These findings hold great promise for the creation of surfaces and materials that are specifically designed to influence biological reactions and show potential for a range of biomedical uses.

Keywords: Cell proliferation, Porosity, Biomedical application, Alamar blue, fluorescence microscopy, breast cells

Introduction

Breast cancer represents a significant global health challenge (Wilkinson & Gathani, 2022). It is a leading health concern among women due to its high mortality and morbidity rate (Kashyap et al., 2022). As a prevalent form of cancer, early diagnosis, efficient treatment, and effective preventative measures are necessary. Implantable systems for drug delivery can overcome the drawbacks of conventional breast cancer treatment methods by enhancing treatment efficacy through targeted therapy and regulated drug release. Therapeutic devices like temporary implants and three-dimensional scaffolds used in tissue engineering are most effective when created with biodegradable polymers (Song et al., 2018). The intricacies involved in cellular responses to biomaterials emphasize the critical importance of meticulously designing, synthesizing, selecting and fabricating biomaterials to ensure the final product mimics biological systems (Abdul-Al et al., 2020). Thus, before approval of biomaterials for medical use, they must adhere to specific criteria and regulatory standards. Among these, biocompatibility stands out as a crucial and fundamental requirement (Nair & Laurencin, 2007; Pişkin, 1995). Many factors affect how tissue responds to an implant; these include the form and composition of the implant as well as the chemical, physical, and biological characteristics of the materials employed (Nair & Laurencin, 2007).

However, most biomedical devices utilized in breast cancer treatment and detection are deleterious to healthy cells. sadly, almost all studies on breast cancer have focused primarily on the destruction or suppression of malignant cells, with little attention to normal breast cells around the tumor site. Understanding the interaction of normal human breast cells with biomaterial surfaces is important for biomedical applications of biomaterials. The present study aims to evaluate the behavior of MCF10A cells when exposed to porous poly-di-methyl-siloxane (PDMS) structures. This study holds lots of promise for a wide range of biological uses, such as tissue engineering, stem cell therapy, and the design of drug delivery and medical devices. PDMS, known for its interaction with living tissues, is widely utilized in the biomedical industry due to its durability (Yirijor et al., 2022).

Moreover, PDMS is an elastomeric material with exceptional mechanical characteristics that make it ideal for a wide range of specialized applications. This material has the qualities of chemical inertness, thermal stability, and ease of handling. It exhibits consistent and uniform properties, capable of reproducing submicron details essential for crafting microstructures (Miranda et al., 2022). Additionally, PDMS provides flexibility for accurate modifications in molecular interactions. Its surface is highly hydrophobic when it initially forms, but it can be made hydrophilic by applying different treatments, like oxygen plasma treatment, UV ozone radiation, self-assembled monolayer coating, or polymer/peptide grafting (Zhang et al., 2013). In this context, the surface of PDMS was modified by creating porous surfaces of different dimensions, and the work has significant potential in oncology research.

Materials and Methods

Preparation and characterization of PDMS substrate

A silicone elastomer curing agent and Sylgard 184 silicone elastomer base were mixed in a 10:1 volume ratio to make PDMS substrates. To achieve consistency, the mixture was vigorously agitated. Degassing was done without the application of heat using an isotemp vacuum oven (model 280A, Themofisher, USA) linked to a high vacuum Edwards pump (E2M265503, Albany, NY) and set at 25 inHg equivalent for an hour without the application of heat. The polymer mixture was mixed with sugar granules of various sizes to create pores of various

dimensions (0-150, 150-250, and 250-500 µm). The blends were evenly spread across twelve-well plates, gently swirled to cover the well surfaces, and left to degas. Subsequently, they were allowed to cool down to room temperature after undergoing a four-hour curing process at 60°C. Surgical blades were utilized to extract the PDMS structures. Following this, the samples were soaked in double-deionized water (DDW) for several days to eliminate the sugar particles. The material was subsequently characterized using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) (IRSpirit, Shimadzu, Kyoto, Japan) (Beer-Lech et al., 2022).

Cell culture

The American Type Culture Collection (ATCC) in Virginia, USA, is the source of the human normal breast cell line MCF-10A, which was cultured in compliance with ATCC protocols. The cells were cultured in DMEM/F12 media supplemented with 0.5 µg/ml hydrocortisone, 5% horse serum, 10 µg/ml insulin, 30 ng/ml murine epidermal growth factor, 100 ng/ml cholera toxin, at 37°C in 5% carbon (iv) dioxide (CO_2) incubator.

Assessment of Cell Proliferation

The Alamar blue assay was used to assess the proliferation of the cells at 4, 48, and 96 h using a microplate reader (Perkin Elmer, Waltham, MA), and the percentage reduction in Alamar blue was determined.

Fluorescent imaging

The study involved the fluorescence staining of MCF 10A cells cultured on the PDMS substrates after varying durations, namely 4, 48, and 96 h. Before each study, the medium from the incubated cells was fully aspirated before fluorescent staining of the cells, and DPBS was used to rinse the cells to eliminate any residual serum. Then, after fixing the cells for 10 minutes with 4% paraformaldehyde, they were rinsed with DPBS. Triton X100 was used to make the cell membranes permeable for 15 minutes, followed by another rinse with Dulbecco's Phosphate-Buffered Saline (DPBS). To prevent non-specific binding, a 1% bovine saline albumin (BSA) solution was applied as a blocking agent, left for 30 minutes, and rinsed with DPBS. Then, Alexa Fluor 555 Rhodamine Phalloidin (Thermo Fisher Scientific, USA) was used to stain the actin cytoskeleton for 30 minutes. Thermo Fisher Scientific's SlowFade Gold Antifade Mountant with 4′,6-diamidino-2-phenylindole (DAPI) (Product # S36938) was also used to stain the cell nuclei on the substrates for 10 minutes. Next, the samples were placed on glass slides and imaged using X 10 and 20 and objectives of a Nikon Ts2R-FL inverted fluorescence microscope, outfitted with a Nikon DS-Fi3 C camera (Nikon Instrument, Inc., Melville, NY) (Nikkhah et al., 2010).

Data analysis

The data was presented as percentage Alamar blue reduction, and to compare multiple groups at the same time point, a one-way analysis of variance (ANOVA) was conducted using Statistical Package for the Social Sciences (SPSS) version 20. The least significant differences were identified at $p < 0.05$.

Results

PDMS Characterization

When PDMS was characterized using FTIR, distinctive peaks that reflect its molecular structure were observed as seen in Figure 1. The stretching vibrations of the methyl (-CH3) groups in the PDMS structure are represented by peaks of 2958.41094 cm^{-1}. Peaks at around 1256.07713 cm^{-1} represent the methyl (-CH3) groups in the PDMS molecule's bending vibrations. Strong peaks at 1009.42407 cm^{-1} are indicative of the silicon-oxygen-silicon (Si–O–Si) backbone of PDMS stretching vibrations. Peaks at 7779.8799 cm^{-1} are representative of the silicon-carbon (Si–C) bonds in PDMS stretching vibrations.

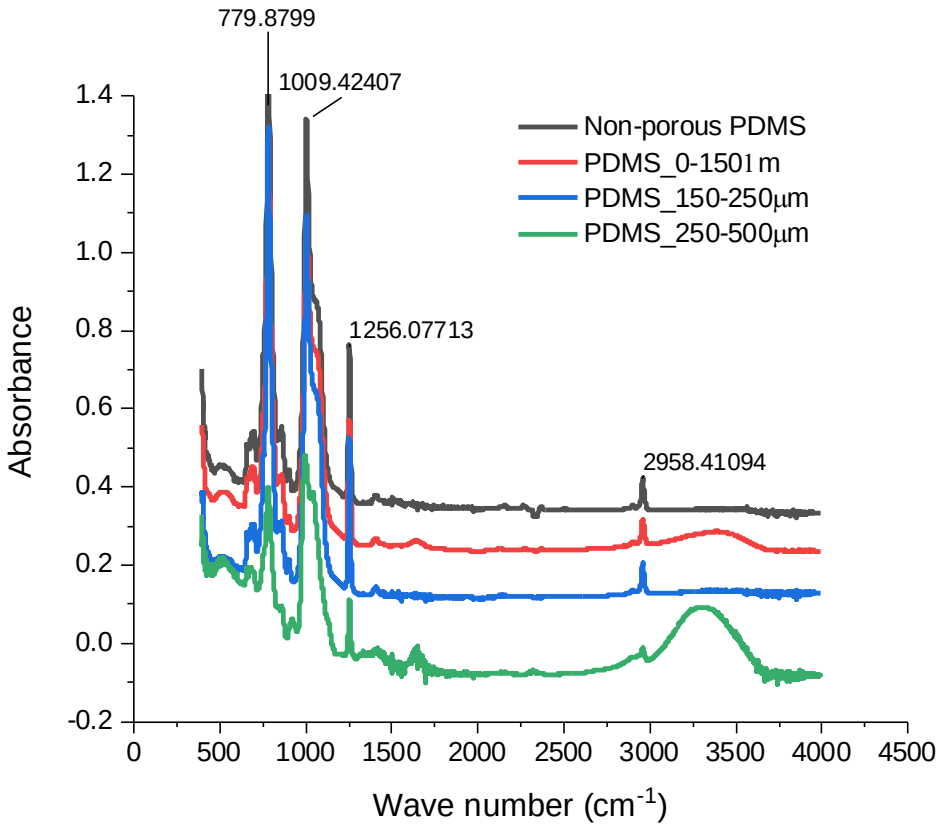


Fig 1: FTIR spectra of non-porous PDMS and PDMS substrate of different pore sizes

Cell proliferation

The effects of PDMS porosity on the metabolic process of MCF10A cells are presented in Figure 2. The results of the percentage Alamar blue reduction indicate no significant difference ($P>0.05$) among all the groups at 4 h post-incubation. However, at 48 and 96 h, a significant increase ($P<0.05$) in the percentage of Alamar blue reduction was evident in the PDMS_250-500 µm group compared to the others. Furthermore, during these time intervals (48 and 96 h), all porous groups exhibited significantly higher ($P<0.05$) Alamar blue reduction in comparison to the non-porous PDMS substrate. Additionally, a clear observation was made regarding a significant reduction ($P<0.05$) in the percentage of Alamar blue as the porosity increased.

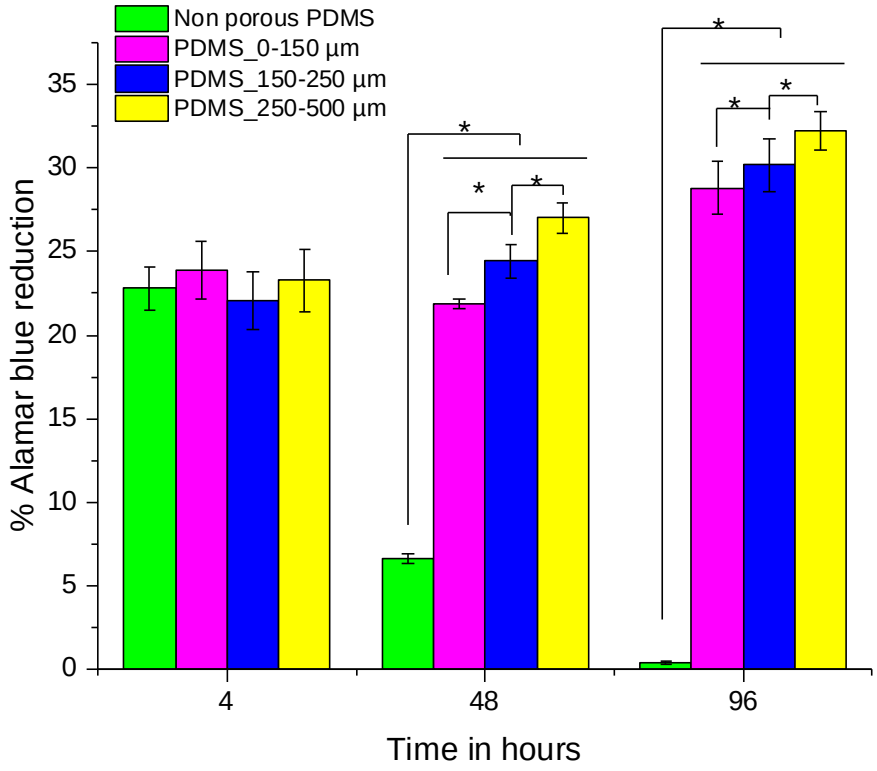
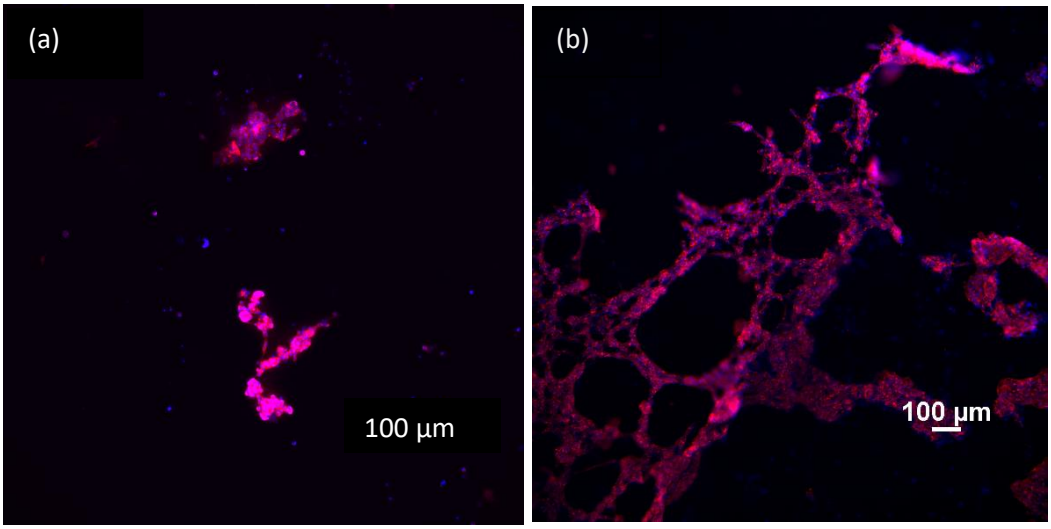


Fig 2: Effect of porosity on percentage Alamar blue reduction at times 4, 48, and 96 h

Fluorescence imaging

The presented fluorescence images captured the behavior of MCF-10A cells when cultivated on PDMS-based surfaces, as illustrated in Figure 3. Notably, there was a substantial enhancement in cell growth as the porosity of the PDMS substrate increased, as evident in the images (Fig. 3b-d). However, the observation revealed a significant decline in cell growth when these cells were cultivated on non-porous PDMS surfaces, as depicted specifically in Figure 3a.



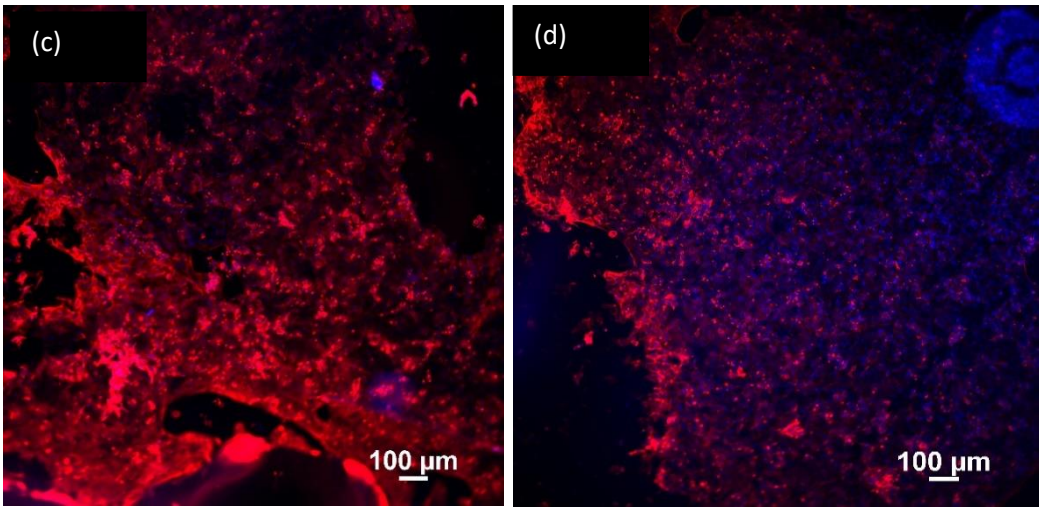


Fig 3: Fluorescence microscopy images of MCF10A cells on PDMS substrate after 96 h demonstrating the effect of porosity on the interaction between MCF10A cells and PDMS surfaces (a) Non-porous PDMS (b) PDMS_0-150µm (c) PDMS_150-250µm (d) PDMS_250-500µm.

Discussion

Material characteristics (such as porosity, pore size, interconnectivity, and fiber diameter) can influence the activity of cells (Loh & Choong, 2013; Zhou et al., 2023). The results of this study, as shown in Figure 2, provide significant novel insights into the impact of PDMS porosity on MCF10A cell proliferation. At 4 h post-incubation, no significant variations ($P>0.05$) were seen in the percentage of Alamar blue reduction across all groups. This implies that, in the early phases, the metabolic response is consistent across different PDMS porosities. However, at 48 and 96 h, there was a noticeable change in metabolic activity, as evidenced by a significant rise ($P<0.05$) in the percentage of Alamar blue reduction in the PDMS_250-500 µm group in comparison to the other groups (Fig. 1). This significant increase in cell proliferation points to the distinctive effect of this specific porosity range on the metabolic processes occurring within cells throughout a prolonged incubation time. A different study showed that chondrocytes had a greater propensity for proliferation and the production of extracellular matrix (ECM) when seeded on scaffolds with pore sizes between 250 and 500 µm (Lien et al., 2009).

Furthermore, at these latter time intervals (48 and 96 h), a consistent pattern was observed. In comparison to the non-porous PDMS substrate, all porous groups showed considerably higher ($P<0.05$) Alamar blue decrease (Fig.1). This finding suggests that PDMS substrates' porous properties may be essential for increasing cellular metabolic activity. The proportion of Alamar blue reduction significantly decreased ($P<0.05$) as porosity increased. Porosity and metabolic activity have an inverse relationship that suggests an ideal porosity range or possible threshold where MCF10A cell metabolism may be best stimulated or facilitated. Porosity and surface area are correlated, and as porosity increases, the scaffold material's surface area progressively improves, which could facilitate the inward growth of more cells or aid in the transportation of nutrients and oxygen since cells are more likely to stick to the scaffold material's surface (Cychosz et al., 2017). An earlier report showed that the impact of 3D silk fibroin frameworks on the growth and movement of human foreskin fibroblast cells indicated that as the pore sizes in the scaffolds expanded, there was a corresponding rise in cell proliferation (Loh & Choong, 2013).

Cell adhesion is the process by which cells adhere to their extracellular matrix through interactions between ligands and integrins (Murphy & O'Brien, 2010). The behavior of MCF-10A cells is

correlated with the porosity of PDMS-based surfaces, as seen in Figure 3, which highlights the crucial role surface properties play in cellular response and adhesion. Higher porosity levels in the PDMS substrate were shown to substantially enhance cell growth, indicating that surface texture has a significant impact on cellular adhesion and proliferation. This finding may have applications in regenerative medicine and tissue engineering, where scaffold materials with different porosities are utilized to promote tissue regeneration and cell proliferation. It highlights how various material designs can either encourage or prevent cellular adhesion and growth depending on the needs. On the other hand, the notable decrease in cell development on non-porous PDMS surfaces highlights the significance of surface roughness in creating an environment that supports cellular adhesion and growth. This information could have a significant impact on the creation of better medical devices or coatings by helping to design biomedical implants or surfaces where managing cell adherence is crucial.

Conclusion

In conclusion, the result of the study implies that certain porosity levels, especially in the PDMS_250-500 µm range, and porous structures may have a substantial effect on cellular metabolic processes. These findings hold great promise for the creation of surfaces and materials that are specifically designed to influence biological reactions and show potential for a range of biomedical uses.

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