

Enhancing Biomedical Applications: Modifying porous poly-dimethyl-siloxane (PDMS) structures with magnetite nanoparticles (MNPs) to Improve Interaction with Normal Human Breast Cells (MCF10A Cells)

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Abstract

The phenomenon of cell-biomaterial interaction is responsible for adherent cells' adhesion to the biomaterial surface and the corresponding cell activities. The study aimed to enhance biocompatibility and versatility by modification of porous PDMS structures with MNPs for their safe interaction with normal human breast cells, MCF10A cell line. Preparation of the MNP-modified porous PDMS substrate was carried out by mixing a silicone elastomer base with a curing agent at a specific ratio, typically in a 10:1, followed by modification with MNP and the creation of pores of different dimensions. The substrate was subsequently characterized with Fourier-transform infrared spectroscopy. Furthermore, the assessment of cell proliferation and fluorescence imaging was done using the Alamar blue assay and fluorescence microscopy, respectively. The result of the study showed that PDMS + MNP (non-porous) did not significantly differ in percentage Alamar blue reduction at 4 hours when compared to PDMS + MNP_0-150, PDMS + MNP_150-250, and PDMS + MNP_250-500. Additionally, all the groups

differed significantly ($P>0.05$) from one another at 48 and 96 h, except the PDMS+MNP_150-250 group compared to the PDMS+MNP_250-500 group, which did not exhibit any significant differences ($P>0.05$). The result further showed that when compared to all other groups, the fluorescence imaging result revealed that, after 96 h, there was very little cell attachment and proliferation on the surface of PDMS+MNP (non-porous). Other groups demonstrated discernable cell adhesion and proliferation over time. These outcomes demonstrate the significance of porosity in influencing cellular interactions and highlight its role in cell proliferation on biomaterial.

Keywords: Cell Surface, Magnetite Nanoparticles, Polydimethylsiloxane, Breast Cell, Proliferation

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Introduction

No cancer type affects women more frequently like breast cancer (Yin et al., 2020). Breast cancer encompasses various stages: early noninvasive types like ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS), primary invasive breast cancer that infiltrates the nearby breast tissue, and advanced or metastatic breast cancer, which involves spreading to lymph nodes or distant organ (Reilly, 2007). Although it has been thoroughly and widely studied by researchers across the world, the incidence and mortality rates are still rising. However, there is a need for novel and innovative methods to improve treatment strategies. In recent times, attention has shifted to implantable biomaterial devices since they have become extremely adaptable drug delivery systems that can provide novel, more potent, and imaginative approaches to the fight against cancer (Chew & Danti, 2017; Veselov et al., 2022). The primary goal of developing drug delivery vehicles is to transport medications to the site of targeted therapeutic action while minimizing unfavorable side effects and effectively addressing delivery-related issues with traditional drug delivery (Senapati et al., 2018). In addition, biomedical technologies are widely used in modern times and are essential to the recovery of biological functions (Ilker et al., 2022; Vogler, 2013).

Moreover, PDMS-based biomaterials have attracted a lot of attention in the biomedical sphere due to their versatile applications, notably in delivering anticancer drugs (Miranda et al., 2022; Potrich et al., 2019; Zhang et al., 2013). Great optical and mechanical characteristics make PDMS a great elastomer for a variety of applications (Miranda et al., 2022). However, there is a need to improve PDMS surfaces to

control their interaction with components of living systems in a manner that mimics the normal physiological state. The purpose of this study was to enhance the biocompatibility and versatility by modification of porous poly-di-methyl-siloxane (PDMS) structures with magnetite nanoparticles (MNPs) to augment their interaction with normal human breast cells, MCF10A cell line. Biomaterial surface crafting is crucial because it directly affects how cells engage with the materials. The results of tissue engineering studies and the efficacy of biomedical implants are largely determined by this interaction (Chen, 2016). Surface modification is a process of altering the surface of materials to enhance their properties. The surface of biomaterials-tissues interactions is essential in drug delivery, cell attachment, proliferation, and tissue regeneration (Vogler, 2013). The significance of this research lies in its potential to revolutionize cancer treatment applications.

Materials and Methods

This study was carried out in the Bioengineering complex of Worcester Polytechnic Institute, Worcester, Massachusetts, USA.

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, and magnetite nanoparticles (MNP) were added. 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. The mixes were distributed uniformly into twelve well plates, gently swirled to coat the bases of the wells, and allowed to degas again. The samples were then allowed to cool to room temperature after being cured for four hours at 60 °C. Then, surgical blades were used to obtain the PDMS+MNP structure. Thereafter, the samples were soaked for several days in double-deionized water (DDW) 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 (CO₂) incubator.

Assessment of Metabolic Activities

The Alamar blue assay was used to test the metabolic activity of cells at 4, 48, and 96 h. Fluorescence intensities were determined at 544 nm using a microplate reader (Perkin Elmer, Waltham, MA), and the percentage reduction in Alamar blue was determined (Zachari et al., 2014).

Fluorescent imaging

The study involved the fluorescence staining of cells 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 Dulbecco's

Phosphate-Buffered Saline (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 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 ten minutes. Subsequently, the samples were mounted on glass slides and imaged using a Nikon Ts2R-FL inverted fluorescence microscope equipped with a Nikon DS-Fi3 C camera (Nikon Instrument, Inc., Melville, NY) and 20 and 10 objectives (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+MNP Characterization

Figure 1 shows the FTIR of the porous PDMS+MNP substrate. Infrared (IR) peaks are seen in the porous PDMS+MNP at the following ranges: 784.8699 cm⁻¹ (involving Si-C stretching and -CH₃ rocking in Si-CH₃), 1006.57259 cm⁻¹ (signaling Si-O-Si stretching), 1258.92861 cm⁻¹ (connected to CH₃ deformation in Si-CH₃), and 29662.68817 cm⁻¹ (signaling asymmetric CH₃ stretching in Si-CH₃).

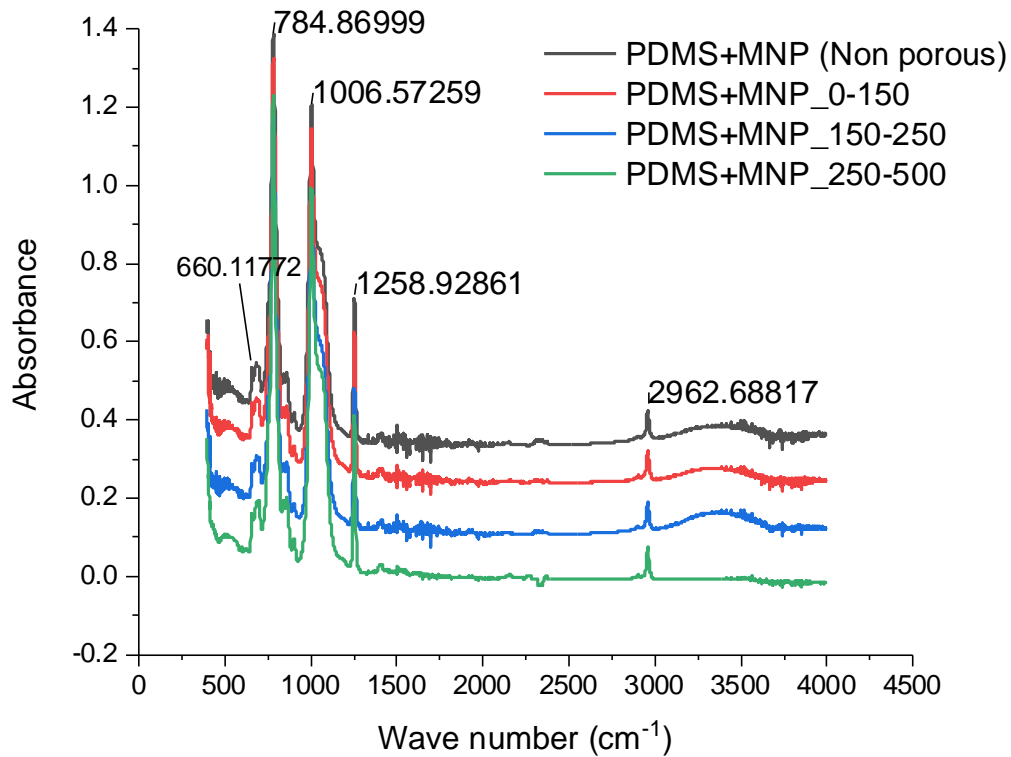


Fig 1: FTIR spectra of PDMS+MNP substrate of different pore sizes

Percentage Alamar Blue Reduction

Figure 2 shows the result of the effect of PDMS+MNP porosity on the percentage of Alamar blue reduction at times 4, 48, and 96 h. At 4 h post-seeding, there was no significant difference ($P>0.05$) in the percentage of Alamar Blue reduction in the non-porous PDMS + MNP group compared to the porous groups, PDMS + MNP_0-

150, PDMS + MNP_150-250, and PDMS + MNP_250-500. However, 48 and 96 h later, significant variations ($P<0.05$) were observed when all the groups were compared, except the PDMS + MNP_150-250 and PDMS + MNP_250-500 groups, where the comparison between these two groups revealed no significant variation ($P>0.05$).

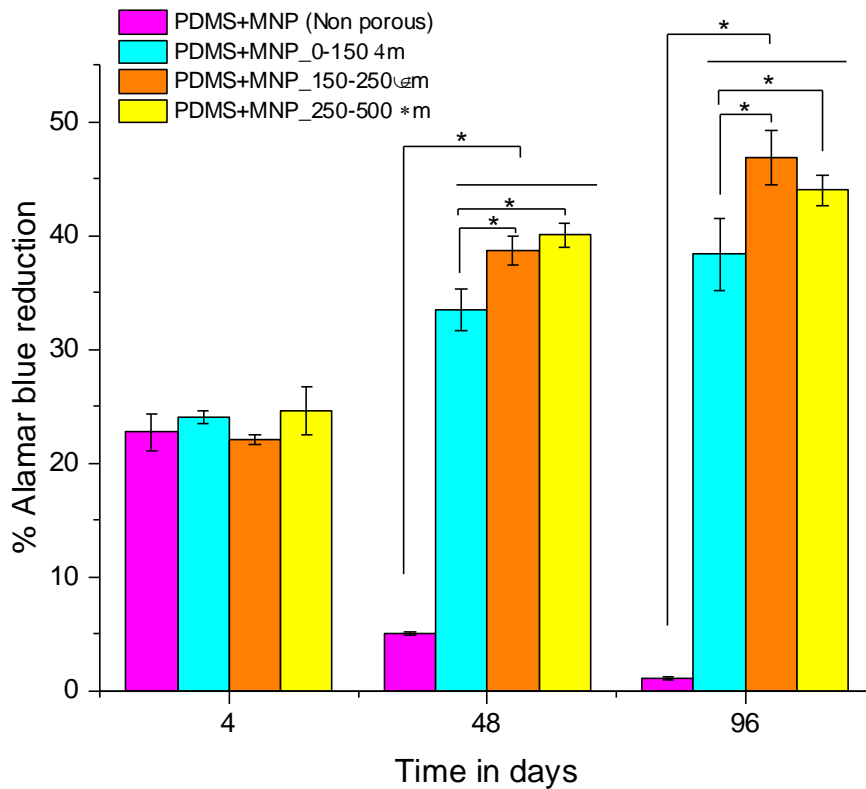
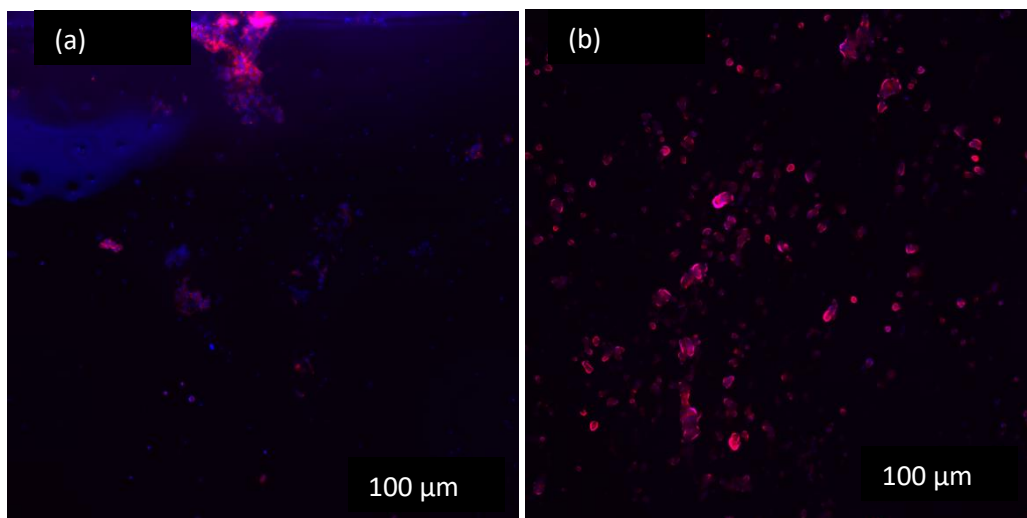


Fig 2: Effect of PDMS+MNP porosity on percentage Alamar blue reduction at times 4, 48, and 96 h

Fluorescence imaging

Figure 3 shows the fluorescence microscopy images of MCF10A cells on PDMS+MNP substrate after 96 h demonstrating the effect of surface modification and porosity on the interaction with MCF10A cells. The fluorescent imaging results revealed a distinctive trend in cellular behavior

among the various groups studied. Notably, in the PDMS+MNP (non-porous) group, there was an observed limited cell proliferation and attachment to the surface compared to all other groups at 96 h. In contrast, the remaining groups demonstrated discernible patterns: as the particle size of the PDMS and MNP increased, a proportional increase in both cell proliferation and attachment was evident.



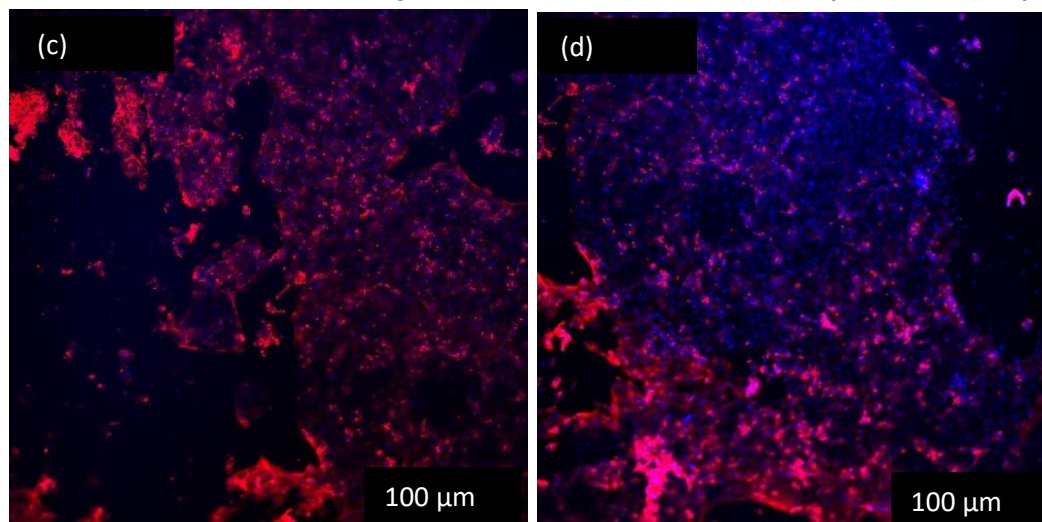


Fig 3: Fluorescence microscopy images of MCF10A cells on PDMS+MNP substrate after 96 h demonstrating the effect of porosity on the interaction with MCF10A cells (a) Non-porous PDMS+MNP (b) PDMS+MNP_0-150 μm (c) PDMS+MNP_150-250 μm (d) PDMS+MNP_250-500 μm .

Discussion

The phenomenon of cell-biomaterial interaction is responsible for adherent cells' adhesion to the biomaterial surface and the corresponding cell activities. This process is inherently complex, including a wide range of physicochemical phenomena that take place at different scales, ranging from the molecular to the organelle level (Sanz-Herrera & Reina-Romo, 2011).

The findings illustrated in Figure 2 offer remarkable observations on how PDMS+MNP porosity affects the percentage of Alamar Blue reduction over time. Initially, at 4 h, there was no significant difference ($P > 0.05$) in the percentage of Alamar Blue decrease between the groups classified by specific particle size ranges (PDMS+MNP_0-150, PDMS+MNP_150-250, and PDMS+MNP_250-500) and the non-porous PDMS+MNP group. This implies that, upon initial cell seeding, the cellular response to these porous substrates of different dimensions did not indicate discernible differences. Significant differences in the percentages of Alamar Blue reduction were noted at 48 and 96-h intervals for every group, with one notable exception of the PDMS+MNP_150-250 compared with the PDMS+MNP_250-500 group. The fact that these two groups showed no discernible difference when directly compared suggests a continuous similarity in cellular response throughout the 48-96-h timeframe. Studies have highlighted how surface modification and material porosity

variations can profoundly influence cellular adhesion, proliferation, and overall behavior (Zhang et al., 2013). These findings have important implications for tissue engineering applications and biomaterial design. To modify biomaterials to elicit desirable cellular behaviors, it is essential to comprehend how varying material porosities and surface modifications affect cellular responses over time. This has implications for several fields including drug delivery systems, biomedical device development, and regenerative medicine.

Furthermore, Figure 3 presents fluorescence microscopy images of MCF10A cells on PDMS+MNP substrates after 96 h. According to earlier research, surface modification treatment is necessary to improve the PDMS surface's hydrophilicity for the best cell adherence (Ye et al., 2014). Furthermore, alterations in the physical characteristics of PDMS have resulted in variations in the adhesion, proliferation, and in-vitro plasticity of cells (Chuah et al., 2020). The PDMS+MNP (non-porous) arrangement significantly ($P < 0.05$) showed decreased cell adhesion and growth. This behavior points to a possible less favorable nature of the non-porous PDMS+MNP surface for cell attachment and proliferation. In contrast, there was a corresponding increase in cell attachment and proliferation in the groups with larger particles within the PDMS+MNP matrix. By offering more locations for cell attachment and signaling cues, studies have repeatedly demonstrated that larger

particle sizes or rougher surface textures can improve cell adhesion, proliferation, and overall interaction with biomaterial surfaces. The distribution of nutrients and the elimination of waste products are limited when holes are too small because cells cannot move inward toward the center of the construction (Murphy & O'Brien, 2010).

Conclusions

Cellular responses are changing, as evidenced by the change in the Alamar blue reduction between the PDMS+MNP groups at 48 and 96 h. Moreover, different cellular behaviors were revealed by fluorescence microscopy, which emphasized the limited cell attachment on non-porous PDMS+MNP surfaces and the correlation between larger particle sizes and increased adhesion and proliferation of cells. These findings demonstrated the essential functions that porosity and particle size play in determining cellular interactions.

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