

Enhanced Mesenchymal Stem Cell Survival and Proliferation on Biomimetic Substrates

Zohreh Montaseri

Shiraz University of Medical Sciences

Fakhrossadat Farvadi

farvadi@gmail.com

Shiraz University of Medical Sciences

Mohammad Javad Raee

Shiraz University of Medical Sciences

Haniyeh Najafi

Shiraz University of Medical Sciences

Mozhgan Abedanzadeh

Shiraz University of Medical Sciences

Ali Mohammad Tamaddon

Shiraz University of Medical Sciences

Article

Keywords: Mesenchymal Stem Cells (MSCs), Cell Imprinting, Biomimetic Substrate, Cell Membrane Coating

Posted Date: July 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10385382/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Controlling the in vitro behavior of mesenchymal stem cells (MSCs) remains a major challenge in regenerative medicine. In this study, we sought to develop an advanced biomimetic culture substrate by integrating complementary physical and biological cues. Fibroblast-imprinted polydimethylsiloxane (PDMS) substrates were fabricated to provide a pseudo-three-dimensional (pseudo3D) topographical cue and subsequently coated with a polydopamine (PDA) layer and fibroblast-derived cell membrane (CM) fragments. Compared with the uncoated or single layer-coated imprinted substrates, the combined CM-PDA coating markedly enhanced the surface hydrophilicity and coating stability. MSCs cultured on CM-PDA-imprinted (CM-PDA-IMP) substrates demonstrated superior cellular performance, including high viability (> 95%), increased proliferation, and enhanced cell spreading over a 72-hour culture period, as confirmed by MTT and live/dead assays. Collectively, these results indicate that the integration of PDA-mediated surface adhesion and native biomimetic membrane coatings with cell shape-imprinted topographies creates a favorable microenvironment that effectively supports MSC survival and proliferation by recapitulating key features of the native cellular niche.

1. Introduction

Stem cells are highly responsive to their surrounding environment, making them valuable for regenerative medicine as well as for studying biological processes such as drug screening and cytotoxicity assessment. Their unique capacity for self-renewal and differentiation is determined not only by their intrinsic genetic makeup but also by a complex set of external cues derived from the surrounding niche.[1] However, maintaining and directing stem cell behavior in vitro remains a major challenge. In addition to their native in vivo environment, stem cells lose many of the biochemical and biophysical signals that regulate their fate.[2] In particular, interactions with neighboring cells and the extracellular matrix (ECM) play crucial roles in controlling cell behavior, including adhesion, growth, proliferation, and differentiation.[2, 3] Therefore, various strategies have been developed to mimic the native cellular niche and provide key regulatory signals such as growth factors, intercellular communication, and ECM-mediated interactions.[1] Among these cues, the physicochemical properties of the culture substrate are especially important. Mesenchymal stem cells (MSCs) can sense and respond to substrate composition, topography, roughness, and mechanical stiffness, which in turn regulate essential biological functions, including gene expression and lineage commitment.[4, 5] Indeed, a previous study has shown that stem cell behavior varies significantly when cells are cultured on hydrogel substrates with different stiffness values.[4] Thus, engineering the physical features of culture surfaces has emerged as an effective approach for modulating cell morphology, function, and fate.[6, 7]

Although three-dimensional (3D) culture systems can better mimic native tissue architecture, their practical limitations have encouraged the development of alternative strategies based on surface engineering. In particular, the modification of substrate topography has attracted attention for creating more favorable environments for stem cell growth. However, identifying the most appropriate surface

pattern for a given cell type remains challenging because of the wide diversity of possible topographies.
[8]

Feeder-layer systems represent another established strategy for supporting stem cell culture in vitro. In these systems, differentiated cells provide a supportive matrix for stem cell attachment and growth while also supplying soluble factors and biomechanical cues, such as topographical features and substrate stiffness.[9, 10] Despite their effectiveness, feeder layers have several limitations, including the risk of feeder-cell carryover, poor reproducibility, and possible contamination with animal-derived pathogens.[1, 10] To overcome these issues, cell-free systems such as Matrigel, which contains a mixture of ECM proteins, have been widely used for stem cell culture. Matrigel has important drawbacks, including high cost, batch-to-batch variability, and potential biosafety concerns.[10]

In addition to biochemical interactions, the presence of neighboring cells in the environment also impacts cell shape and behavior through the regulation of the cytoskeleton and membrane-mediated interactions.[1, 11] The fluid nature of the cell membrane allows membrane proteins to reorganize dynamically according to cellular needs and establish functional interactions with adjacent cells.[12] Therefore, the complete elimination of cell-derived structural and membrane-associated cues may reduce the physiological relevance of in vitro culture systems, increase the doubling time and reduce the size of colonies.[10] In agreement with this concept, Joddar et al. demonstrated that fixed feeder cells can serve as a cost-effective alternative to live feeder layers for large-scale cultivation of human induced pluripotent stem cells by preserving structural features while eliminating biological activity.[10] Similarly, we have previously shown that culturing cells on "pseudo3D" substrates can promote the adoption of more natural cell morphology.[11]

Based on these considerations, the present study aimed to develop a biomimetic culture substrate for MSCs by combining topographical and membrane-derived biological cues. Fibroblast-imprinted substrates were designed to preserve the cellular morphology and provide a bioinspired surface topography capable of generating mechanical signals. These substrates were further coated with fibroblast-derived cell membranes to facilitate interactions with stem cell receptors and deliver biologically relevant surface cues. We then investigated the effects of this biomimetic bed on MSC adhesion, proliferation, and viability in vitro.

2. Results and discussion

In the complex in vivo microenvironment, cells are continuously exposed to a wide range of biochemical and mechanical signals. Like biochemical cues, physical signals can also trigger diverse cellular responses. Previous studies have demonstrated that matrix-derived mechanical signals, such as surface topography, can strongly impact cellular behaviors such as growth, proliferation, differentiation, motility, migration, and gene expression through their effects on cytoskeletal organization and nuclear architecture.[11, 13] McNamara et al. emphasized the importance of cell surface topography and shape in maintaining the cellular architecture and mechanical signal transduction.[14] Surface topography also

plays a central role in cell adhesion and cytoskeletal reorganization, which in turn affects intracellular tension and the responsiveness of mechanosensitive ion channels. These changes can alter cellular metabolism, intracellular signaling pathways, and ultimately gene expression patterns.[15] The present study was designed to fabricate and evaluate fibroblast-imprinted biomimetic substrates as pseudo3D culture platforms for MSCs. The substrates were functionalized with PDA to improve surface wettability and cell membrane attachment, followed by coating with fibroblast-derived cell membrane fragments to provide bioactive interfacial cues. Their physicochemical properties and effects on MSC morphology, viability, and proliferation were then investigated.

2.1 Preparation of biomimetic beds (pseudo3D substrates)

2.1.1 Cell imprinting

Cell imprinting is intended to reproduce cell-like topographical cues on the substrate surface, which may influence subsequent stem cell behavior through contact guidance and mechanosensitive interactions. [16] To this end, fibroblasts were selected as template cells because of their well-defined spindle-shaped morphology with an average size of approximately 18 μm , ease of culture, and relevance as stromal cells that naturally contribute structural and biochemical support within tissue microenvironments.[17, 18]

These cells were cultured on standard polystyrene culture plates according to standard cell culture conditions until they reached 70–80% confluency before imprinting.

PDMS was chosen as the molding polymer for cell imprinting because of its favorable properties, including biocompatibility, optical transparency, flexibility, ease of handling, and ability to reproduce micro- and nanoscale features.[19] In addition, its density is close to that of cell culture medium, which minimizes mechanical compression of the cells during molding.[20] After curing, the polymeric mold was gently removed from the plate surface and cleaned with a sodium hydroxide solution to eliminate residual cellular material and chemical contaminants. Optical microscopy revealed clear and well-defined cell-like patterns on the polymer surface, confirming successful transfer of the fibroblast morphology to the PDMS substrate (Fig. 1).

The high fidelity of the imprints, characterized by distinct cellular voids and negligible residual debris, confirms the efficient transfer of the biological interface to the elastomeric mold. This finding indicates that the observed patterns represent the morphological and topographical footprint of the cells at the micro- and nanoscale rather than artifactual cellular material. [22] These findings are consistent with established principles of soft lithography, where substrate compliance facilitates high-resolution replication of nanoscale membrane features. [23] Our results corroborate prior evidence that cell-mediated imprinting effectively reproduces complex geometries on biocompatible elastomers [24, 25], extending our previous work on cell-imprinted ‘pseudo3D’ substrates. [11]

2.1.2 Cell membrane coating of imprints

The use of cell membrane fragments was intended to provide biologically relevant surface-associated cues, including membrane proteins and lipid components, that may better mimic aspects of the native cellular microenvironment than purely synthetic coatings.[26] PDMS is widely used for the fabrication of patterned biomaterials because of its stability and compatibility; however, its intrinsic hydrophobicity can hinder cell adhesion and surface biofunctionalization.[27, 28] Various methods, including PEG coating [29], ECM protein coating [30], and plasma treatment [31], have been employed to increase the hydrophilicity of PDMS. Nevertheless, these approaches often face challenges such as instability, hydrophobic recovery, or procedural complexity. PDA offers an attractive alternative because dopamine can undergo self-polymerization under alkaline conditions to form a stable and biocompatible nanoscale coating. This layer improves surface energy, wettability, and cellular interactions. [32]

As shown in Fig. 2, the unmodified imprinted PDMS substrate (IMP) was initially smooth and hydrophobic. After PDA coating, the surface became darker, rougher, and more hydrophilic. These changes are consistent with the known physicochemical characteristics of PDA and are important because they can substantially affect subsequent membrane attachment and cell–surface interactions. [33]

The ATR-FTIR spectrum of IMP (Fig. 2.b) showed characteristic PDMS absorption bands at 789 cm^{-1} assigned to the Si–C stretching vibration, 1011 cm^{-1} attributed to the Si–O–Si symmetric stretching vibration, 1257 cm^{-1} corresponding to CH_3 deformation in Si– CH_3 , and 2963 cm^{-1} associated with asymmetric CH_3 stretching of the side chains.[34] In the PDA-IMP spectrum, an additional peak at 1614 cm^{-1} was observed, which can be attributed to N–H bending of amide groups, supporting successful deposition of the PDA layer.[35, 36]

PDA is known to improve cell adhesion by enhancing surface wettability and promoting protein adsorption, thereby facilitating cell attachment.[37] Recent studies have also shown that PDA coatings can improve cell behavior on various substrates.[38, 39] However, given the potential influence of the PDA concentration on the stability of subsequent cell membrane coatings [40, 41], optimization of the PDA concentration is crucial for developing a reliable surface modification strategy for stable MSC culture. To address this issue, IMP substrates were coated with dopamine at concentrations ranging from 0 to 10 mg/mL for 24 h, and subsequent cell membrane adhesion was assessed via fluorescence microscopy following eosin staining (Fig. 3). These fluorescence findings indicate relative membrane retention on the substrate after washing, although they do not by themselves establish the structural organization or functional integrity of the coated membrane layer. We observed increased membrane adhesion with increasing PDA concentration up to 5 mg/mL, after which adhesion decreased. This finding strongly suggests that PDA promoted membrane retention on the IMP surface but that this effect strongly depended on the coating concentration. At PDA concentrations below 5 mg/mL, surface hydrophilicity was likely insufficient to support stable membrane retention on the IMP surface. Conversely, PDA concentrations above 5 mg/mL may have rendered the surface excessively hydrophilic, thereby reducing the adsorption of membrane-associated components. Among the concentrations

tested, 5 mg/mL PDA produced the highest CM coating efficiency. The improved membrane retention may be attributed to the presence of PDA amine- and catechol-containing functional groups, which can enhance interfacial interactions and modulate surface wettability. [42] The PDA coating of PDMS has also previously been reported to support MSC adhesion and maintenance of multipotency, making it a useful strategy for mechanobiological studies.[43]

2.1.3 Surface topography

Surface roughness can influence protein adsorption, focal adhesion formation, and cell spreading, thereby affecting how cells interact with engineered substrates. [44] AFM was utilized to analyze the roughness and topographical characteristics of the modified imprint substrates. The unmodified IMP substrate exhibited a relatively smooth morphology (Fig. 4.a), with a root mean square roughness (Rq) of 13.57 ± 4.22 nm (Table 1), and showed no apparent irregularities. After PDA coating, the surface roughness markedly increased to 87.45 ± 28.32 nm, likely due to the aggregation of PDA during polymerization [45] and the formation of PDA nanoparticles on the PDMS surface through a self-assembly process. [46] After subsequent coating with cell membrane fragments, the Rq of CM-PDA-IMP decreased to 29.29 ± 14.36 nm, indicating partial smoothing of the PDA-coated surface by the membrane layer. The increase in roughness observed for CM-IMP relative to bare IMP also suggests successful deposition of the membrane-derived material on the patterned surface, although the effect was less pronounced than that produced by the PDA coating.

Table 1
Roughness parameters of differently coated substrates measured by AFM

Roughness Parameter	IMP	CM-IMP	PDA-IMP	CM-PDA-IMP
Arithmetic roughness (Ra, nm)	1.82 ± 0.44	12.13 ± 0.35	18.26 ± 1.41	1.746 ± 0.1
MS roughness (Rq, nm)	13.57 ± 4.22	34.77 ± 11.36	87.45 ± 28.32	29.29 ± 14.36
Maximum height (Rz, nm)	21.84 ± 4.17	58.04 ± 28.317	174.24 ± 31.53	81.56 ± 25.95

2.1.4 Surface wettability

Because initial cell attachment is strongly influenced by interfacial wettability and protein adsorption, these changes in contact angle are likely related to the improved MSC viability and growth observed on the modified substrates.[47] Surface wettability plays a crucial role in cell–material interactions.[43, 48] Surface modification strategies that increase hydrophilicity often improve biological interactions;[49] however, excessive hydrophilicity may impair initial attachment and delay cell spreading.[50] Research has shown that highly hydrophilic surfaces may impair the initial adsorption of proteins required for cell

attachment. Because protein adsorption mediates integrin-dependent cell adhesion [50, 51], appropriate tuning of hydrophilicity is essential for ensuring the effective attachment of CM fragments and because protein adsorption mediates integrin-dependent cell adhesion. [50]

The surface hydrophilicity of the IMP, CM-IMP, PDA-IMP, and CM-PDA-IMP substrates was evaluated via water droplet contact angle measurements (Fig. 4.c and d). Compared with the bare IMP, CM-IMP, PDA-IMP, and CM-PDA-IMP all presented reduced contact angles (θ), which CM-PDA-IMP presented the lowest contact angle ($\theta = 107.5 \pm 0.28$, $94.27 \pm 0.85^\circ$, $88.53 \pm 1.05^\circ$, and $\theta = 59.11 \pm 1.68^\circ$ respectively). These findings indicate that the combined PDA and CM coating was more effective than either modification alone in improving surface wettability. Previous studies have shown that unmodified hydrophobic IMP surfaces can induce the rupture of CM vesicles and the formation of a monolayer, whereas hydrophilic surfaces are more favorable for bilayer membrane formation. On hydrophobic PDMS, vesicle rupture and fusion are driven by interfacial energy differences between the substrate and the vesicle suspension, resulting in the formation of a lipid monolayer. Thus, the increased hydrophilicity of PDA-modified substrates may also contribute to improved membrane organization and retention on the patterned surface [52, 53]. Importantly, a lower contact angle alone does not necessarily predict superior biological performance; rather, an appropriate balance between hydrophilicity, surface chemistry, and biological functionality is needed.[52, 53]

2.2 Investigating the effects of biomimetic beds on mesenchymal stem cells

Because MSCs are highly sensitive to both topographical and biochemical microenvironmental cues, they represent an appropriate model for evaluating the biological performance of engineered biomimetic substrates.[54] After fabrication and surface modification, the substrates (CM-IMP and CM-PDA-IMP) were placed in 12-well culture plates with the patterned surface facing up to serve as a biomimetic bed for MSC culture.

2.2.1 MSC morphology

After 24 hours of culture on the biomimetic beds, the MSCs were fixed and examined via fluorescence microscopy and SEM (Fig. 5). The cells grown on standard culture plates appeared more elongated and spread, whereas the cells cultured on biomimetic beds showed reduced flattening and elongation and adopted a more three-dimensional morphology. This observation is consistent with previous research. [11] The less flattened morphology observed on the imprinted substrates may indicate reduced adaptation to conventional two-dimensional spreading and a closer approximation of cell behavior in structured microenvironments.

For the CM-PDA-IMP substrate, interpretation of the cell morphology via SEM was more difficult because drying-induced cracking of the PDA-containing coating interfered with visualization of the intact cell outlines. Therefore, while the substrate appeared supportive of cell growth, detailed morphological

assessment of this surface by SEM was limited, and complementary imaging approaches would be useful in future studies.

2.2.2 Cell viability

The biocompatibility of the PDA and CM coatings was first assessed via the MTT assay. The viability of the MSCs cultured on CM-PDA-IMP was greater than 95%, which was comparable to that observed on standard tissue culture plates, whereas the viability of the cells cultured on the IMP substrate was lower (Fig. 6.a). CM coating alone did not markedly increase cell viability. These findings suggest that combined PDA and CM modification provided a more favorable microenvironment for MSC survival than the imprinted substrate alone. PDA likely improved surface wettability and promoted initial attachment, whereas the membrane coating may have provided biologically relevant interfacial cues that supported cell survival. Similar observations have been reported in a study by Hung et al. [55], in which dopamine-coated PDMS surfaces resulted in only limited improvement in MSC viability (less than 2%) compared with unmodified PDMS, indicating that PDA alone may not be sufficient and may perform better when combined with additional bioactive surface components. The improved performance of CM-PDA-IMP may therefore be attributed to the complementary roles of its two components: PDA enhances the physicochemical suitability of the surface, whereas the cell membrane layer contributes biomimetic biological signals that more closely resemble the native stem cell microenvironment.

To further investigate the effects of different substrates on MSC viability and growth, live/dead staining was performed. Dead cells were identified by PI staining and red fluorescence, whereas live cells were stained with AO, and emitted green fluorescence (Fig. 6.b). Microscopic examination during the first 24 h of culture revealed that the MSCs proliferated on CM-PDA-IMP, with predominantly green fluorescent cells and no obvious evidence of cytotoxicity. In addition, a greater number of cells were observed on CM-PDA-IMP than on the other tested substrates. After 72 h, the cell density on CM-PDA-IMP remained greater than that on CM-IMP and standard plates. Therefore, the live/dead results indicated that the biomimetic substrate did not induce notable cytotoxicity and was supportive of MSC attachment and proliferation.

The lower cell density observed on CM-IMP may reflect less favorable surface properties in the absence of PDA, whereas the CM-PDA-coated substrate provided a more supportive interface for cell spreading and expansion. These observations are in agreement with previous reports showing lower cell density on unmodified PDMS than on PDA-coated substrates. [28, 46] The enhanced survival and proliferation of MSCs on CM-PDA-IMP are likely due to the combined effects of the PDA layer and the membrane coating. The PDA coating improved the interfacial wettability and supported protein adsorption and early cell adhesion. PDA has also been shown to directly reduce oxidative stress and downregulate senescence-associated genes. [56] Moreover, the cell membrane layer provides biologically relevant surface cues that may facilitate cell–surface interactions in a more biomimetic manner. [57] Together, these features appear to create a more favorable culture environment for MSC expansion over the 72-h period.

3.2.4 Cell cycle analysis

In cell cycle analysis, DNA-binding fluorochromes generate fluorescence signals proportional to the cellular DNA content, allowing discrimination of cells in the G0/G1, S, and G2/M phases. [58] Compared with cells cultured on standard tissue culture plates, those cultured on the biomimetic substrate presented an increased proportion of cells in S phase together with a reduced G1 duration (Fig. 7). An increased proportion of cells in S phase generally reflects greater entry into DNA synthesis and is commonly interpreted as an indicator of active cell cycle progression. [59] This finding suggests that the biomimetic bed promoted cell cycle transitions and supported a more proliferative cellular state. The shortened G1 phase implies a more rapid transition to DNA synthesis, which is consistent with the higher cell density and viability observed in the MTT and live/dead assays.

Taken together, these results indicate that the biomimetic substrate provided a more supportive microenvironment for MSC expansion than did conventional culture surfaces. By integrating topographical, physicochemical, and biological cues, the substrate may better mimic aspects of the native ECM niche and thereby mitigate the “culture shock” often associated with standard two-dimensional culture conditions.

Despite the promising outcomes observed in this study, several limitations should be acknowledged. First, the stability of the bio-functionalized substrates was evaluated within a 72 h window; future research should explore the long-term durability and mechanical integrity of the coating under extended culture conditions. Second, while a PDA concentration of 5 mg/mL was identified as optimal, the dose-dependent effects of the cell membrane fragment density on MSC attachment and viability warrant further systematic investigation. Furthermore, this study focused primarily on morphological and metabolic viability; comprehensive genetic and proteomic characterization—such as the expression of stemness markers or lineage-specific genes—is necessary to fully elucidate the influence of this biomimetic platform on MSC phenotype maintenance.

3. Conclusion

This study describes the development and validation of a biomimetic substrate designed to better regulate the in vitro microenvironment of MSCs. By integrating topographical, chemical, and biological cues, a pseudo3D culture platform was created that improved MSC adhesion, viability, and proliferation. The substrate was fabricated through a multistep process in which PDMS was first imprinted with an NIH/3T3 fibroblast morphology to reproduce cell-like topographical features. This imprint substrate was then coated with PDA to reduce the hydrophobicity of the PDMS and provide a favorable interface for subsequent biofunctionalization. Among the tested conditions, a PDA concentration of 5 mg/mL enabled the most effective attachment of fibroblast-derived cell membrane fragments, yielding the CM-PDA-IMP substrate.

MSCs cultured on this biomimetic substrate exhibited a more 3D-like morphology and high viability (> 95% in the MTT assay) and improved growth over 72 h with minimal cytotoxicity. These findings suggest that the combined use of PDA and a cell membrane coating created a more supportive and biomimetic interface than either unmodified or singly modified substrates.

In recent years, increasing evidence of how cells sense and respond to substrate properties has enabled more rational design of culture platforms that regulate the cellular phenotype and function.[4, 15, 60] In this context, the present findings further support the view that conventional two-dimensional monolayer systems do not adequately reproduce the complexity of native three-dimensional biological environments.[61] Therefore, the combination of cell imprinting, PDA functionalization, and cell membrane coating represents a promising approach for the development of advanced stem cell culture platforms, with potential applications in MSC expansion, in vitro microenvironment engineering, and regenerative medicine.

4. Methods

4.1 Materials

Paraformaldehyde, acridine orange (AO), propidium iodide (PI), and phenylmethanesulfonylfluoride (PMSF) were purchased from Sigma–Aldrich, USA. Bradford reagent and ethanol were obtained from Merck, Germany. Polydimethylsiloxane (PDMS; Sylgard® silicone elastomer) was purchased from Dow Corning, USA. The NIH/3T3 cell line was obtained from the Iran Pasteur Institute. Cell culture media, including Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), were from Shellmax, Iran. Human umbilical cord mesenchymal stem cells (hUCMSCs) were provided by the Center for Nanotechnology in Drug Delivery (SUMS).

4.2 Preparation of biomimetic beds (pseudo3D substrates)

4.2.1 Cultivation of template cells

NIH/3T3 mouse normal fibroblasts were utilized as template cells to generate imprints reflecting fibroblast morphology and topography. The cells were cultured in DMEM in 12-well plates for 24 h under standard culture conditions until they reached approximately 70–80% confluency.

4.2.2 Cell imprinting

PDMS was used to mold the template cells. [4] After the culture medium was removed, the cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) to preserve the cellular morphology and membrane structure. [15] PDMS and the curing agent were mixed at a 10:1 weight ratio and poured onto the fixed cells at room temperature until the surface of each well was fully covered with an approximate polymer thickness of 1 mm. The plates were kept at room temperature to allow trapped air bubbles formed during mixing and pouring to escape. They were then transferred to an incubator at 37°C to speed up and complete the curing process. After overnight curing, the cell-imprinted

PDMS substrates were detached from the plate surface. To remove cell debris and chemical residues, the molds were immersed in 1 M NaOH for at least 1 hour and then thoroughly washed with deionized water until a neutral pH was reached. The cell imprints were examined via optical microscopy and atomic force microscopy (AFM) (see section 2.1.5).

4.2.3 Cell membrane isolation

NIH/3T3 fibroblasts cultured in cell culture plates were collected and centrifuged at 700 g for 5 min. The cells were washed with precooled PBS at 4°C, pH 7.4, and centrifuged at 600 g for 5 minutes. The cell pellets were subsequently resuspended in hypotonic lysis buffer (20 mM Tris-HCl buffer, pH 7.4; 2 mM MgCl₂; 10 mM KCl; and 1 mM EDTA-free PMSF (protease inhibitor)). The cell suspension was incubated on ice for 15 minutes, followed by repeated rapid freeze–thaw cycles to disrupt the cells. The cell nuclei were removed via centrifugation at 700 g for 10 minutes at 4°C. The resulting supernatant was further centrifuged at 22,000 g for 30 minutes to isolate CMs. [62] The protein content of the isolated CMs was quantified via the Bradford dye-binding assay.

4.2.4 Coating PDMS imprints with the cell membrane

To increase the hydrophilicity of the PDMS surface and facilitate cell membrane attachment, the imprinted substrates were precoated with a thin PDA layer. Briefly, imprinted PDMS substrates (IMPs) were immersed in dopamine hydrochloride solutions at different concentrations (0.2, 0.5, and 1% w/v in 10 mM Tris-HCl buffer, pH 8.5) for 24 h. Then, the PDA-coated IMP (PDA-IMP) was rinsed twice with deionized water to remove unbound PDA. [28]

For the cell membrane coating used to prepare the biomimetic bed, IMP and PDA-IMP substrates were immersed in fusogenic buffer composed of 20 mM HEPES buffer, 150 mM NaCl, and 2 mM CaCl₂, adjusted to pH 7.5. [63] Cell membrane fragments (CM) were added to the buffer at a final concentration of 250 µg/mL and incubated overnight at 37°C in a humidified atmosphere containing 5% CO₂.

4.2.5 Characterization of biomimetic beds

ATR-FTIR spectroscopy (Tensor II, Bruker, Germany) was performed to confirm PDA deposition on the imprinted PDMS surface. The absorbance spectra were recorded over the range of 400–4000 cm⁻¹.

The presence of the CM coating was verified via fluorescence microscopy. The CM-IMP and CM-PDA-IMP substrates were fixed with 4% paraformaldehyde for 15 minutes, washed, and stained with 1% eosin for 5 minutes. The samples were subsequently observed under a fluorescence microscope equipped with a green filter set, with excitation/emission wavelengths of approximately 525/546 nm.

The surface topography and roughness of the IMP, CM-IMP, PDA-IMP, and CM-PDA-IMP substrates were analyzed via atomic force microscopy (AFM; Standard, Ara Research, Iran) in tapping mode.

Water contact angle measurements were performed via a CAG-10 dynamic contact angle analyzer to assess changes in surface hydrophilicity following PDA and CM coating. Measurements were performed

using multiple water droplets, and the results were averaged ($n = 3$).

4.3 MSC culture on biomimetic beds

To evaluate the effects of biomimetic beds on MSC morphology, survival, and proliferation, MSCs were cultured on IMP, CM-IMP, and CM-PDA-IMP substrates. Standard polystyrene culture plates were used as controls.

4.3.1 Assessment of cell morphology

Cell morphology on various substrates was examined using scanning electron microscopy (SEM; TESCAN-Vega3, TESCAN) at an accelerating voltage of 20 kV. After removing the culture medium, cells were rinsed with PBS and fixed with 4% paraformaldehyde solution to preserve cellular morphology. After 20 minutes, paraformaldehyde was removed, and the samples were washed with deionized water. Cells were dehydrated using a graded ethanol series (30%, 50%, 70%, 90%, and 96%) for 10 minutes at each concentration. [64] Before imaging, the samples were coated with a thin layer of gold.

4.3.2 MTT assay

The biocompatibility and cytotoxicity of the biomimetic beds toward MSCs were evaluated via the MTT assay. The substrates were placed at the bottom of the wells of 12-well cell culture plates, and the MSCs were seeded at a density of 6×10^5 cells/well and incubated at 37°C in a humidified atmosphere containing 5% CO₂. The cells were then washed with PBS, treated with MTT reagent (0.5 mg/ml), and incubated at 37°C for 4 h. The medium was then removed, and the resulting formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm with a reference wavelength of 650 nm via a microplate reader. Cell viability was determined via absorbance measurements, with values compared to those of cells cultured on standard tissue culture plates (SPL®) used as the control group.

4.3.3 Live/Dead assay

MSCs were cultured on biomimetic beds and control plates for 3 days to assess viability and proliferation. The cells were seeded at a density of 6×10^5 cells/well on each substrate and incubated at 37°C with 5% CO₂. After incubation, the cells were washed with PBS and stained with 5 µg/mL acridine orange (AO) and 5 µg/mL propidium iodide (PI) for 5 minutes. The cells were then washed with PBS, fixed with 4% paraformaldehyde, and examined under a fluorescence microscope (Carl Zeiss, Jena, Germany).

2.2.4 Cell cycle assessment

The DNA content of PI-stained cells was assessed by flow cytometry to quantify the percentage of cells in the G1, S, and G2/M phases. [65, 66] Approximately 2×10^5 MSCs were seeded onto both standard plates and biomimetic substrates and cultured for 72 hours. Following incubation, the cells were harvested, and the resulting pellets were gently resuspended in 500 µL of PBS to obtain uniform single-cell suspensions. For fixation, each cell suspension was transferred into 4.5 mL of ice-cold 70% ethanol

and stored at 4°C for at least 2 hours. The cells were subsequently centrifuged to remove the ethanol, resuspended in 5 mL of PBS, and centrifuged again. The final cell pellet was then incubated in 1 mL of PI staining solution supplemented with 0.1% v/v Triton X-100 and 100 µg/ml DNase-free RNase A for 15 minutes at 37°C in the dark before flow cytometry. Prior to data acquisition, microscopic examination was conducted to ensure the absence of cell aggregation. Subsequently, cell cycle analysis was performed via a FACSCalibur™ flow cytometer (Becton-Dickinson, USA), and the resulting data were analyzed via FlowJo software. The duration of the G1 phase was calculated via the following equation:

Equation (1)

$$TG_1 = \frac{TC}{\ln 2} \times \ln(f_{G_1} + 1)$$

where TC is the cell cycle length, or a doubling time of approximately 40 hours in the study, and where f_{G_1} is the fraction of cells in the G₁ phase.

2.2.4 Image processing

Microscopic image processing was performed using ImageJ (version 1.54g). To improve visual clarity, contrast was adjusted globally across the entire images. Furthermore, the cell boundary in the CM-PDA-IMP SEM panel was manually outlined to specifically delineate the region of interest. No other data manipulations were performed.

Declarations

Acknowledgments

This work is a part of Zohreh Montaseri thesis financially supported by Shiraz University of Medical Sciences (Grant Number 25136).

Author contributions

- Z.M.:** Investigation, Writing – original draft.
- F.F.:** Conceptualization, Methodology, Supervision, Formal analysis, Interpretation, Writing – review & editing.
- M.J.R.:** Supervision, Methodology, Validation.
- H.N.:** Investigation, Data curation, Visualization.
- M.A.:** Resources, Investigation, Validation.
- A.M.T.:** Funding acquisition, Conceptualization, Supervision, Formal analysis, Writing – review & editing.

All authors have read and approved the final version of the manuscript.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. This includes the original microscopic images used for characterization and analysis.

Declaration of competing interest

There are no conflicts of interest to disclose.

Funding Declaration

This research was financially supported by Shiraz University of Medical Sciences Under Grant Number 25136.

References

1. Ueki, M. & Ito, Y. In vitro niches for the culture of pluripotent stem cells. (2019).
2. Çelebi-Saltik, B. & Çelebi-Saltik, B. Extracellular matrix proteins for stem cell fate. *Adv Surf. Stem Cell. Res.* 1–21 (2016).
3. Argentati, C. et al. Insight into mechanobiology: How stem cells feel mechanical forces and orchestrate biological functions. *Int. J. Mol. Sci.* **20**, 5337 (2019).
4. Mahmoudi, M. et al. Cell-imprinted substrates direct the fate of stem cells. *ACS nano.* **7**, 8379–8384 (2013).
5. Fisher, O. Z., Khademhosseini, A. & Langer, R. Peppas, N. A. Bioinspired materials for controlling stem cell fate. *Acc. Chem. Res.* **43**, 419–428 (2010).
6. Shimizu, T., Yamato, M., Kikuchi, A. & Okano, T. Cell sheet engineering for myocardial tissue reconstruction. *Biomaterials* **24**, 2309–2316 (2003).
7. Singhvi, R., Stephanopoulos, G. & Wang, D. I. Effects of substratum morphology on cell physiology. *Biotechnol. Bioeng.* **43**, 764–771 (1994).
8. Xiao, L., Sun, Y., Liao, L. & Su, X. Response of mesenchymal stem cells to surface topography of scaffolds and the underlying mechanisms. *J. Mater. Chem. B.* **11**, 2550–2567 (2023).
9. Chan, S. W., Rizwan, M. & Yim, E. K. Emerging methods for enhancing pluripotent stem cell expansion. *Front. cell. dev. biol.* **8**, 70 (2020).
10. Joddar, B., Nishioka, C., Takahashi, E. & Ito, Y. Chemically fixed autologous feeder cell-derived niche for human induced pluripotent stem cell culture. *J. Mater. Chem. B.* **3**, 2301–2307 (2015).
11. Farvadi, F. et al. Cell shape affects nanoparticle uptake and toxicity: An overlooked factor at the nanobio interfaces. *J. Colloid Interface Sci.* **531**, 245–252 (2018).

12. Yu, C. et al. Integrin-matrix clusters form podosome-like adhesions in the absence of traction forces. *Cell. Rep.* **5**, 1456–1468 (2013).
13. Varedi, M., Ghahary, A., Scott, P. & Tredget, E. Cytoskeleton regulates expression of genes for transforming growth factor- β 1 and extracellular matrix proteins in dermal fibroblasts. *J. Cell. Physiol.* **172**, 192–199 (1997).
14. McNamara, L. E. et al. The role of microtopography in cellular mechanotransduction. *Biomaterials* **33**, 2835–2847 (2012).
15. Mashinchian, O. et al. Cell-imprinted substrates act as an artificial niche for skin regeneration. *ACS Appl. Mater. Interfaces.* **6**, 13280–13292 (2014).
16. Hasannejad, F., Montazeri, L., Mano, J. F. & Bonakdar, S. Regulation of cell fate by cell imprinting approach in vitro. *Bioimpacts* **14**, 29945 (2023).
17. Baker, D. W., Liu, X., Weng, H., Luo, C. & Tang, L. Fibroblast/fibrocyte: Surface interaction dictates tissue reactions to micropillar implants. *Biomacromolecules* **12**, 997–1005 (2011).
18. Marsh, M. N. & Trier, J. S. Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum: I. Structural features. *Gastroenterology* **67**, 622–635 (1974).
19. Miranda, I. et al. Properties and applications of pdms for biomedical engineering: A review. *Funct. Biomater.* **13**, 2 (2021).
20. Nosrati, H., Fallah Tafti, M., Aghamollaei, H. & Bonakdar, S. Moosazadeh Moghaddam, M. Directed differentiation of adipose-derived stem cells using imprinted cell-like topographies as a growth factor-free approach. *Stem Cell. Rev. Rep.* **20**, 1752–1781 (2024).
21. Espósito, A. C. C., Brianezi, G., Miot, L. D. B. & Miot, H. A. Fibroblast morphology, growth rate and gene expression in facial melasma. *An. Bras. Dermatol.* **97**, 575–582 (2022).
22. Ghazali, Z. S. et al. Neural priming of adipose-derived stem cells by cell-imprinted substrates. *Biofabrication* **13**, 035009. 10.1088/1758-5090/abc66f (2021).
23. Muys, J. J. et al. Cellular transfer and afm imaging of cancer cells using bioimprint. *J. nanobiotechnol.* **4**, 1. 10.1186/1477-3155-4-1 (2006).
24. Kavand, H. et al. Cell-imprint surface modification by contact photolithography-based approaches: Direct-cell photolithography and optical soft lithography using pdms cell imprints. *ACS Appl. Mater. Interfaces.* **11**, 10559–10566. 10.1021/acsami.9b00523 (2019).
25. Samsuri, F., Alkaisi, M. M., Evans, J. J., Chitcholtan, K. & Mitchell, J. S. Detection of changes in cell membrane structures using the bioimprint technique. *Microelectron. Eng.* **88**, 1871–1874. <https://doi.org/10.1016/j.mee.2010.12.069> (2011).
26. De Pasquale, D. et al. Homotypic targeting and drug delivery in glioblastoma cells through cell membrane-coated boron nitride nanotubes. *Mater. Des.* **192**, 108742 (2020).
27. Fu, J., Chuah, Y. J., Ang, W. T., Zheng, N. & Wang, D. A. Optimization of a polydopamine (pd)-based coating method and polydimethylsiloxane (pdms) substrates for improved mouse embryonic stem cell (esc) pluripotency maintenance and cardiac differentiation. *Biomater. Sci.* **5**, 1156–1173 (2017).

28. Sharma, D. et al. Polydopamine and collagen coated micro-grated polydimethylsiloxane for human mesenchymal stem cell culture. *Bioact Mater.* **4**, 142–150 (2019).
29. Long, H., Lai, C. & Chung, C. K. Polyethylene glycol coating for hydrophilicity enhancement of polydimethylsiloxane self-driven microfluidic chip. *Surf. Coat. Technol.* **320**, 315–319 (2017).
30. Kefallinou, D. et al. Enhanced and stabilized mesenchymal stem cell growth inside plasma pre-treated and collagen-coated pdms-based microfluidic chambers. *Surf. Coat. Technol.* **466**, 129658 (2023).
31. Neves, L. B. et al. A review of methods to modify the pdms surface wettability and their applications. *Micromachines* **15**, 670 (2024).
32. Lu, M., Ding, L., Zhong, T. & Dai, Z. Improving hydrophilicity and adhesion of pdms through dopamine modification assisted by carbon dioxide plasma. *Coatings* **13**, 126 (2023).
33. Yang, D. H., Jung, S., Kim, J. Y. & Lee, N. Y. Fabrication of a cell-friendly poly (dimethylsiloxane) culture surface via polydopamine coating. *Micromachines* **13**, 1122 (2022).
34. Johnson, L. M. et al. Elastomeric microparticles for acoustic mediated bioseparations. *J. Nanobiotechnol.* **11**, 1–8 (2013).
35. de Campos Vidal, B. & Mello, M. L. Collagen type i amide i band infrared spectroscopy. *Micron* **42**, 283–289 (2011).
36. Luo, H. et al. Facile synthesis of novel size-controlled antibacterial hybrid spheres using silver nanoparticles loaded with poly-dopamine spheres. *Rsc Adv.* **5**, 13470–13477 (2015).
37. Deng, Y. et al. Bioinspired and osteopromotive polydopamine nanoparticle-incorporated fibrous membranes for robust bone regeneration. *NPG Asia Mater.* **11**, 39 (2019).
38. van der Westen, R. et al. Myoblast cell interaction with polydopamine coated liposomes. *Biointerphases* **7** (2012).
39. Lee, J. H., Lim, Y. W., Kwon, S. Y. & Kim, Y. S. In vitro effects of mussel-inspired polydopamine coating on ti6al4v alloy. *J. Tissue Eng. Regen Med.* **10**, 273–278 (2013).
40. Yu, Y. et al. Is polydopamine beneficial for cells on the modified surface? *Regenerative Biomaterials.* **9**, rbac078 (2022).
41. Harati, J. et al. Polydopamine-mediated protein adsorption alters the epigenetic status and differentiation of primary human adipose-derived stem cells (hascs). *Front. Bioeng.* **10**, 934179 (2022).
42. Omidian, H. & Wilson, R. L. Polydopamine applications in biomedicine and environmental science. *Materials* **17**, 3916 (2024).
43. Chuah, Y. J. et al. Simple surface engineering of polydimethylsiloxane with polydopamine for stabilized mesenchymal stem cell adhesion and multipotency. *Sci. Rep.* **5**, 18162 (2015).
44. Majhy, B., Priyadarshini, P. & Sen, A. Effect of surface energy and roughness on cell adhesion and growth—facile surface modification for enhanced cell culture. *RSC Adv.* **11**, 15467–15476 (2021).

45. Du, X. et al. Uv-triggered dopamine polymerization: Control of polymerization, surface coating, and photopatterning. *Adv. Mater.* **26**, 8029–8033 (2014).
46. Xue, P. et al. Surface modification of poly (dimethylsiloxane) with polydopamine and hyaluronic acid to enhance hemocompatibility for potential applications in medical implants or devices. *ACS Appl. Mater. Interfaces.* **9**, 33632–33644 (2017).
47. Xu, L. C. & Siedlecki, C. A. Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces. *Biomaterials* **28**, 3273–3283 (2007).
48. Zhou, P. et al. Rapidly-deposited polydopamine coating via high temperature and vigorous stirring: Formation, characterization and biofunctional evaluation. *PLoS One.* **9**, e113087 (2014).
49. Echeverria Molina, M. I., Malollari, K. G. & Komvopoulos, K. Design challenges in polymeric scaffolds for tissue engineering. *Front. Bioeng.* **9**, 617141 (2021).
50. Xing, Z. et al. Altered surface hydrophilicity on copolymer scaffolds stimulate the osteogenic differentiation of human mesenchymal stem cells. *Polymers* **12**, 1453 (2020).
51. Cai, S. et al. Recent advance in surface modification for regulating cell adhesion and behaviors. *Nanotechnol Rev.* **9**, 971–989 (2020).
52. Ryu, Y. S. et al. Continuity of monolayer-bilayer junctions for localization of lipid raft microdomains in model membranes. *Sci. Rep.* **6**, 26823 (2016).
53. Zhang, Y., Wang, X., Ma, S., Jiang, K. & Han, X. Lipid membrane formation on chemical gradient modified surfaces. *RSC Adv.* **6**, 11325–11328 (2016).
54. Tsai, A. C., Jeske, R., Chen, X., Yuan, X. & Li, Y. Influence of microenvironment on mesenchymal stem cell therapeutic potency: From planar culture to microcarriers. *Front. Bioeng.* **8**, 640 (2020).
55. Hung, H. S. et al. Enhanced biocompatibility and differentiation capacity of mesenchymal stem cells on poly (dimethylsiloxane) by topographically patterned dopamine. *ACS Appl. Mater. Interfaces.* **12**, 44393–44406 (2020).
56. Deng, Z. et al. Biofunction of polydopamine coating in stem cell culture. *ACS Appl. Mater. Interfaces.* **13**, 10748–10759 (2021).
57. Alvarez-Lorenzo, C. et al. Biomimetic cell membrane-coated scaffolds for enhanced tissue regeneration. *Adv. Mater.* **37**, 2507084 (2025).
58. Darzynkiewicz, Z. Critical aspects in analysis of cellular DNA content. *Curr. Protoc. Cytom.* **52** (2), 8 (2010).
59. Laskey, R. A., Fairman, M. P. & Blow, J. J. S phase of the cell cycle. *Science* **246**, 609–614 (1989).
60. Saha, K. et al. Surface-engineered substrates for improved human pluripotent stem cell culture under fully defined conditions. *PNAS* **108**, 18714–18719 (2011).
61. Manthey, C. L. et al. Jnj-28312141, a novel orally active colony-stimulating factor-1 receptor/fms-related receptor tyrosine kinase-3 receptor tyrosine kinase inhibitor with potential utility in solid tumors, bone metastases, and acute myeloid leukemia. *Mol. Cancer Ther.* **8**, 3151–3161 (2009).

62. Zhang, N., Li, M., Sun, X., Jia, H. & Liu, W. Nir-responsive cancer cytomembrane-cloaked carrier-free nanosystems for highly efficient and self-targeted tumor drug delivery. *Biomaterials* **159**, 25–36 (2018).
63. Miller, E., Stubbington, L., Dinet, C. & Staykova, M. Biophysical insights from supported lipid patches. *Adv. Biomembr. Lipid Self-Assembly*. **29**, 23–48 (2019).
64. Heckman, C., Kanagasundaram, S., Cayer, M. & Paige, J. Preparation of cultured cells for scanning electron microscope. PREPRINT (Version 1) available at Research Square [(2007). <https://doi.org/10.1038/nprot.2007.504>]
65. Darzynkiewicz, Z. & Juan, G. DNA content measurement for DNA ploidy and cell cycle analysis. *Curr. Protoc. Cytom.* **7.5** (1-7.5), 24 (1997).
66. Pozarowski, P. & Darzynkiewicz, Z. Analysis of cell cycle by flow cytometry. *Checkpoint Controls Cancer: 2: Activation Regul. Protocols* 301–311 (2004).

Figures

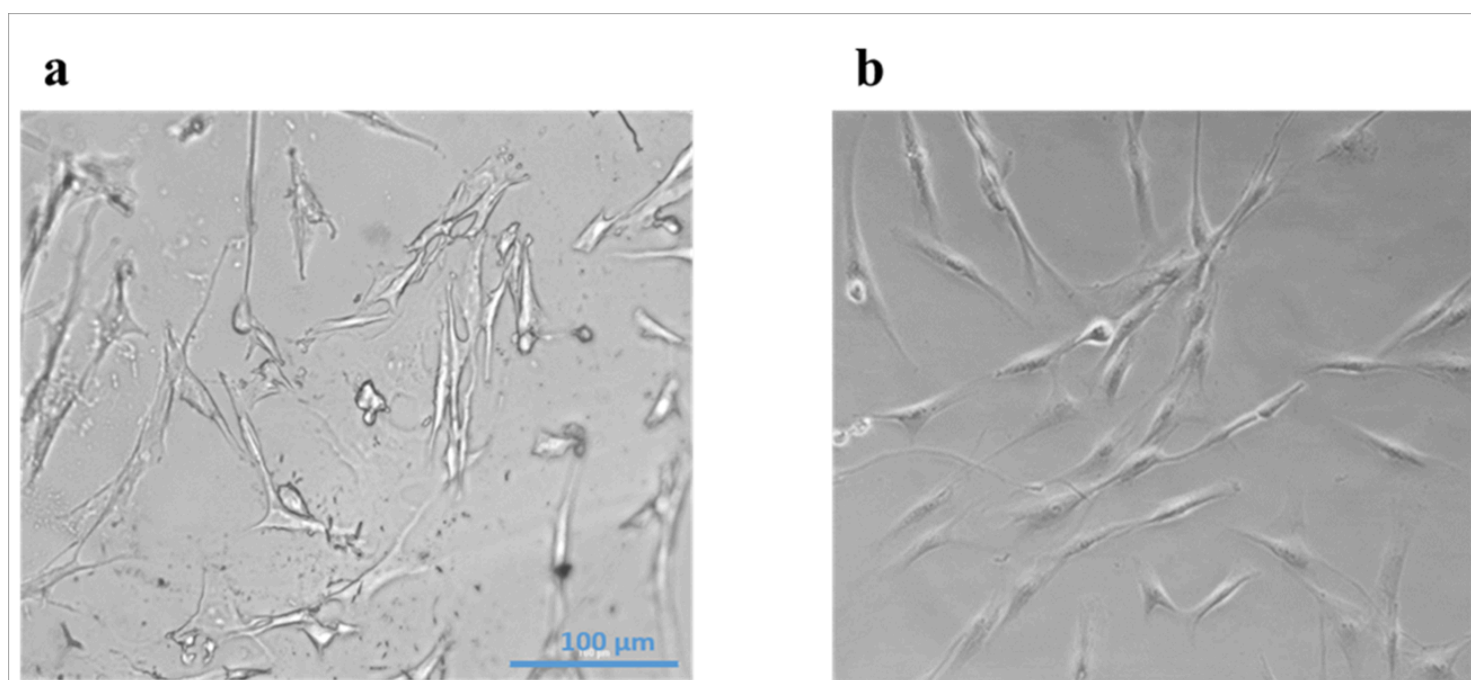


Figure 1

Light microscopy image of the NIH/3T3 fibroblast cell imprint (cell void) (a) and NIH/3T3 fibroblast cell morphology (reprinted with permission from Esposito et al.) [21] (b) (for better resolution, images were taken at a lower cell confluency).

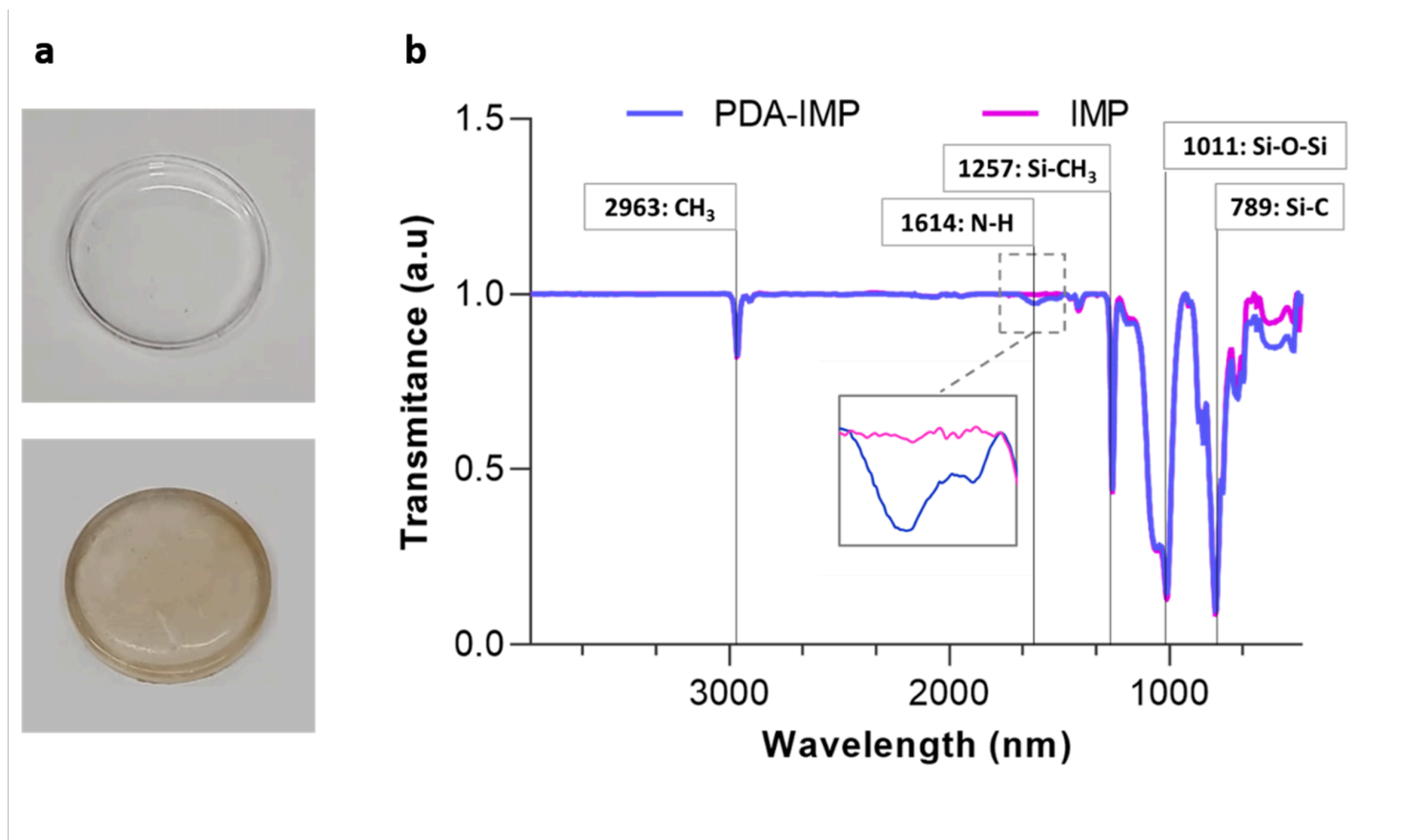


Figure 2

a) PDMS substrate before (top) and after coating with PDA (bottom); b) ATR-FTIR spectra of the PDMS imprint (IMP) and PDA-coated PDMS imprint (PDA-IMP).

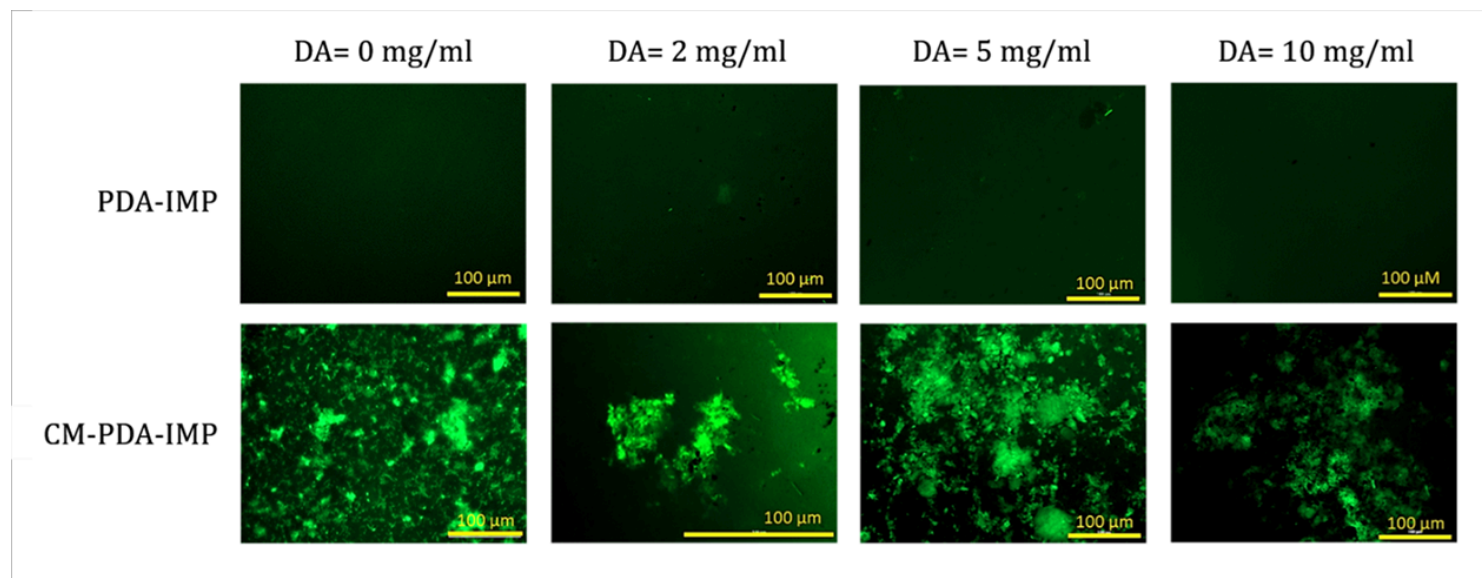


Figure 3

Effect of dopamine (DA) concentration during PDA coating on subsequent cell membrane (CM) attachment to the PDA-IMP substrate. Green fluorescence shows cell mebrane patches on PDA-IMP.

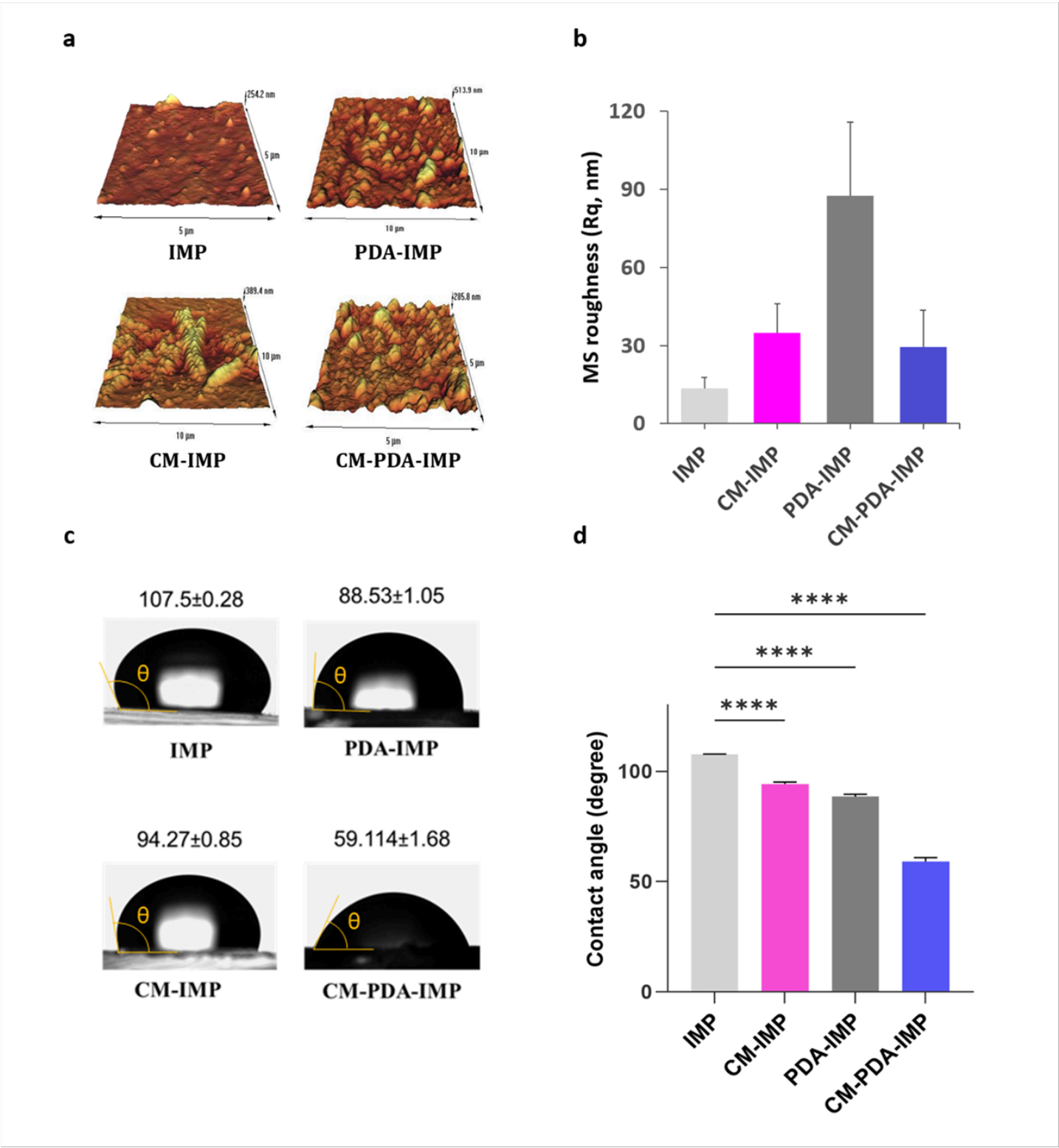


Figure 4

AFM micrographs of various imprint substrates (a); MS surface roughness analysis (b); representative images of water droplet formation on various imprint substrates for wettability analysis (c); and

quantification of water droplet contact angles (d). (IMP: imprint, CM-IMP: cell membrane-coated imprint, PDA-IMP: polydopamine-coated imprint, CM-PDA-IMP: cell membrane-polydopamine-coated imprint)

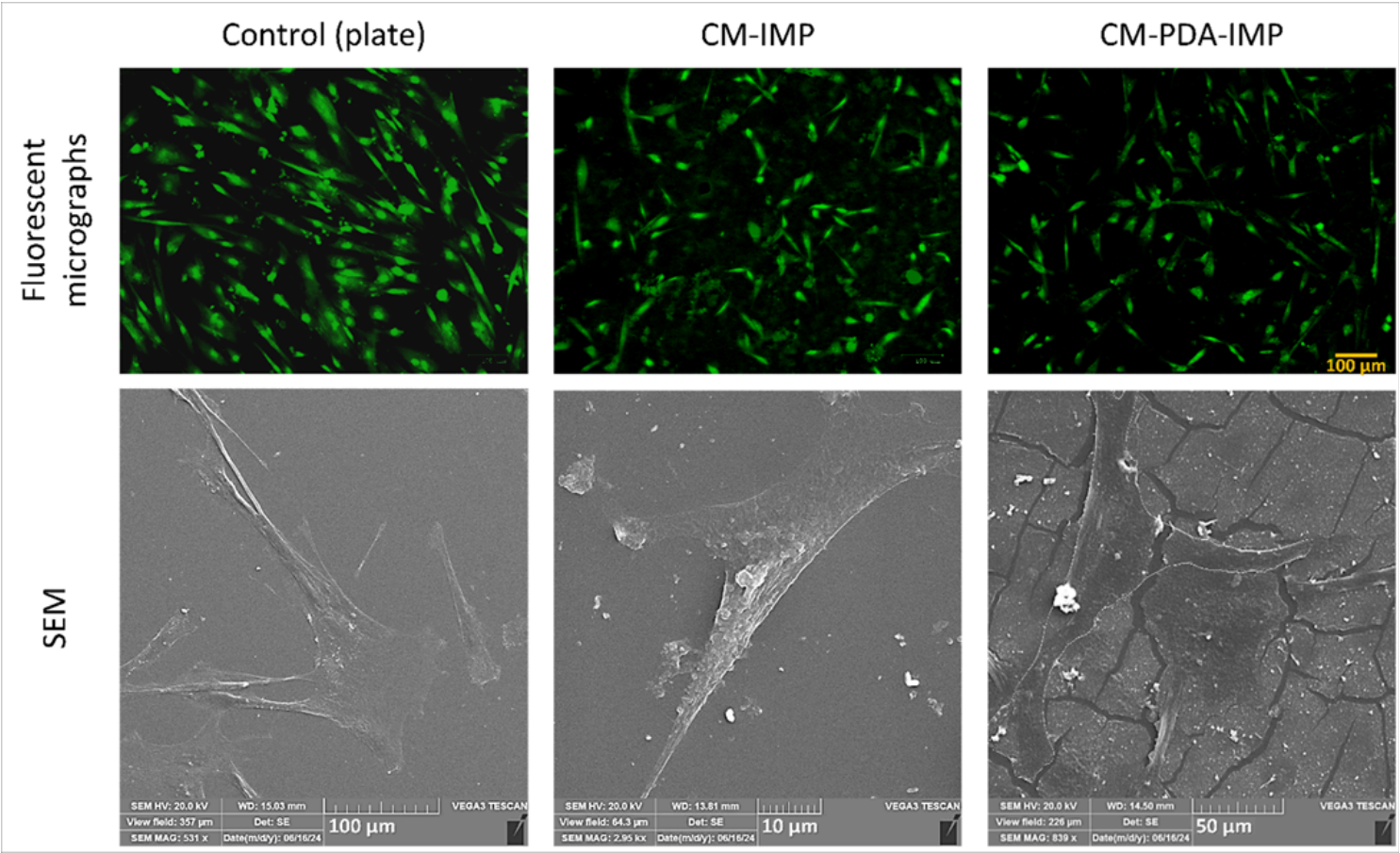


Figure 5

Morphological assessment of MSCs cultured on different substrates. Representative fluorescence images (upper panel) and SEM micrographs (lower panel) of MSCs cultured on control polystyrene plates, CM-IMP, and CM-PDA-IMP substrates. The CM-PDA-IMP surface displays characteristic drying-induced microcracking due to specimen preparation, which partially obscured cellular morphology and introduced deformation artifacts. To improve visual clarity, the cell boundary in the last SEM panel was manually outlined.

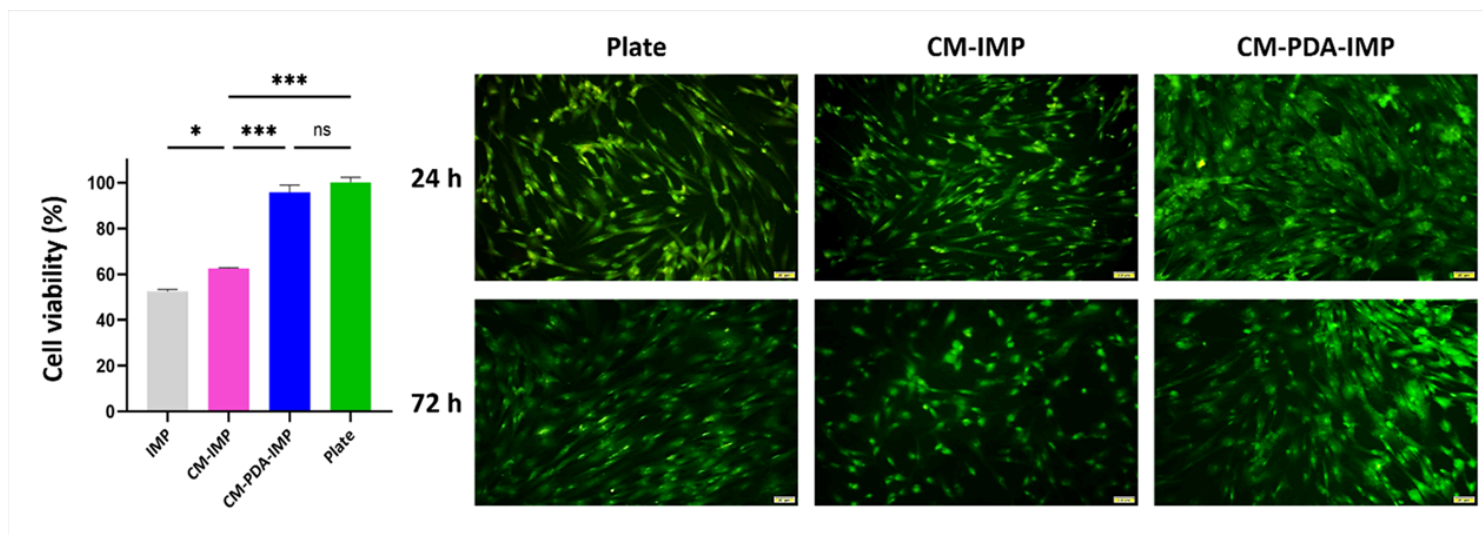


Figure 6

Quantitative analysis of MSC viability via the MTT assay (a) and cytotoxicity analysis via live/dead staining of MSCs cultured on different substrates (b). (IMP: imprint, CM-IMP: cell membrane-coated imprint, PDA-IMP: polydopamine-coated imprint, CM-PDA-IMP: cell membrane-polydopamine-coated imprint)

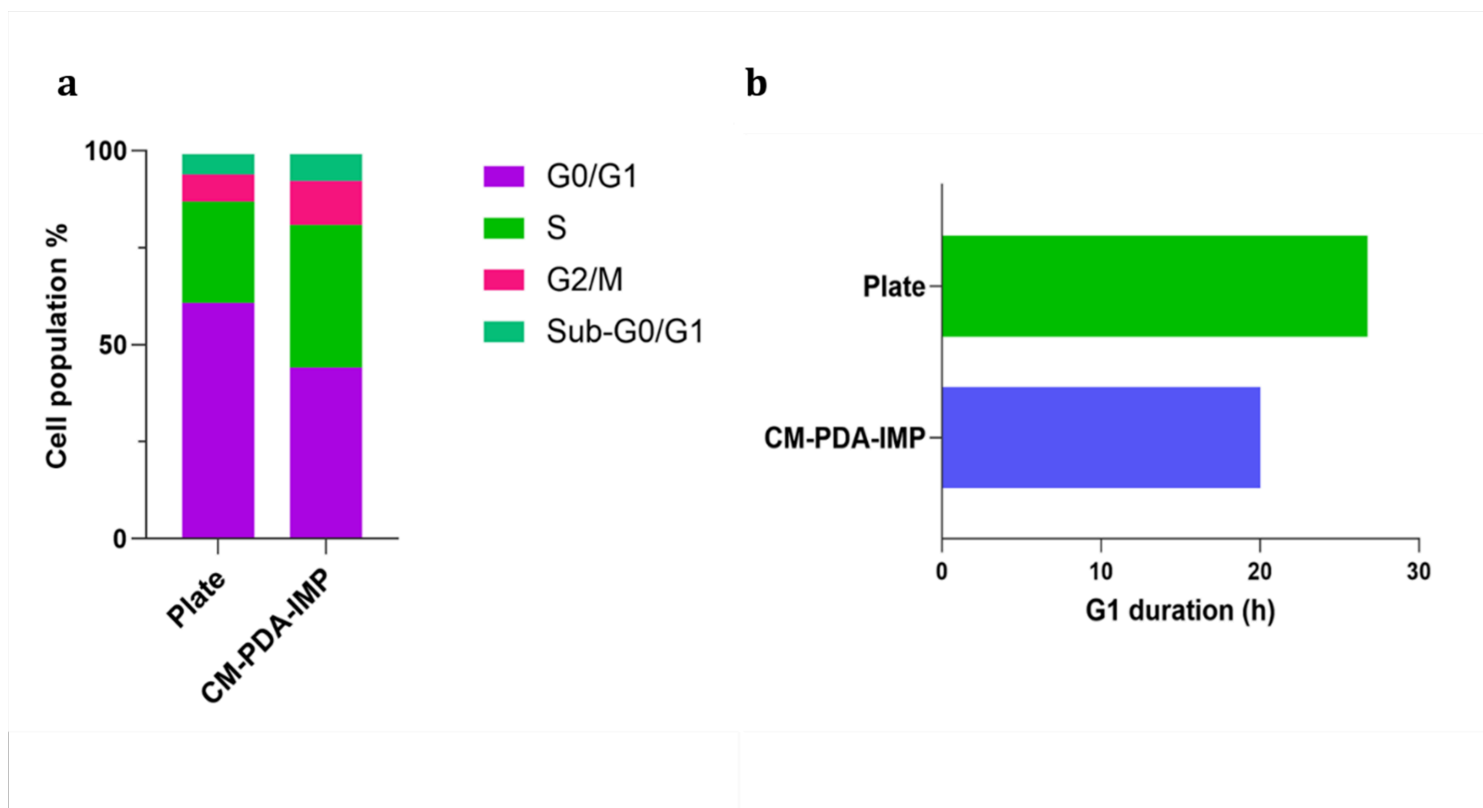


Figure 7

Percentages of MSC populations in different cell cycle phases (a) and G1 durations (h) (b) in MSCs cultured on standard plates and cell membrane-polydopamine-coated-imprinted substrates (CM-PDA-

IMP).