A Study of Interaction Between hWJ-MSCs and SiO₂-Coated PDMS Micropattern

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Abstract. In order to enhance the likelihood of bone healing process from fracture, currently bone graft combined with osteocytes is a new alternative in promoting new bone formation. Silica-based nanoparticles had been proven to induce intrinsic biology activity, especially in promoting bone-formation and differentiation of mesenchymal stem cells towards osteocytes. Previous studies had shown positive results of new bone tissue formation by incorporating SiO₂ nanoparticles synthesized using TEOS. In this current study, the experiment was using micropatterning technique, with PDMS as the substrate, which was coated by SiO₂ nanoparticle powder with the particle size distribution between 150nm-450nm. The effect of SiO₂-coated PDMS on (Wharton’s jelly mesenchymal stem cells) hWJ-MSCs viability was evaluated using MTT cytotoxicity assay for 1, 3, 5, and 7 days, which was compared with two treatments: cells grown with SiO₂ powder without patterns and cells on 96 well plate). The MTT assay result showed that SiO₂-coated PDMS micropattern was non-toxic to the cells and had the highest increase in cell viability for 7 days in comparison to the two controls used.

1. Introduction
Bone fracture is an untoward result and needs to be rapidly healed since it is uncomfortable and diminishes daily movement and productivity. Regeneration of new bone tissue could be improved by the involvement of mesenchymal stem cells that have the capability to differentiate into osteocyte and suitable growth surface for the cells to proliferate and differentiate [1]. Bone graft using certain scaffold or biomaterial is a standard method to provide faster the healing process by serving as a matrix for bone regeneration. However, the bone tissue need cells that could differentiate into osteocytes to produce new bone tissue replacing the existing scaffold. This process could be improved by preparing the osteocytes and then seed the cells into a scaffold [2]. Silica is one important component that could interact with mesenchymal stem cells to further differentiate into osteocytes that could synthesize organic matrix and accumulate bone minerals in the matrix [3].

Currently there are many available methods to differentiate hWJ-MSC into osteocytes, and one of the most recent is by utilizing micropatterning to provide better surface for cell attachment and differentiation. Micropattern is a method to fabricate surface according to the condition in vivo, in which the cells are usually grown on surface with specific topography that could affect cell mechanobiology. There was a report stating that micropatterning with PVA (polyvinyl alcohol) as the basic material could improve faster osteogenesis [4]. Several previous studies have already shown a positive result from growing cells on micropattern coated with SiO₂ to assist in osteogenic differentiation, and most of the studies were doing the research using TEOS as the source of the SiO₂
In this study, PDMS will be used for the micropatterned substrate and will be coated by SiO$_2$ nanoparticle powder with size ranging around 150 – 450 nm, the biocompatibility of the added SiO$_2$ particles micropattern with hWJ-MSCs would be observed from cell morphology and cell viability.

2. Materials and Methods

2.1 Micropattern Fabrication

The schematic process of fabricating a SiO$_2$-Coated PDMS is shown on Figure 1. An acrylic board was laser cut to form a square sized 20x20 mm, with nine rectangular holes sized 1x15 mm (Figure 1a). The pattern was then put into a PTFE mold (Figure 1b) and covered (Figure 1c) with PDMS (Dow Corning) with the curing agent ratio of 12:1 (w/w). The PDMS was cured using an oven at 70°C for 20 minutes. After that, the PDMS was peeled off from the mold (Figure 1d), and the top of PDMS micropattern was swabbed with the same previously mixed liquid PDMS (Figure 1e). The PDMS micropattern was stamped on SiO$_2$ powder (Figure 1f). The area between the pattern, which has an excess of PDMS liquid and SiO$_2$ powder, was cleared manually to prevent any formation of PDMS solid. Lastly, the SiO$_2$-coated PDMS micropattern was cured with an oven at 70°C for 30 minutes, assuring the curing process (Figure 1g).

![Figure 1. The schematic procedure of fabricating a SiO$_2$-coated PDMS](image)

2.2 SiO$_2$ Characterization

SiO$_2$ from Sigma Aldrich is a fumed SiO$_2$ with amorphous structure that was confirmed using X-Ray Diffraction (XRD) and has particle size around 70 – 400 nm with mostly distributed on around 150 – 400 nm that was characterized with Particle Size Analysis (PSA) at Center of Advanced Science Bandung Institute of Technology.

2.3 Surface Morphology

The PDMS surface morphology before and after coating process and the cells attachment on SiO$_2$-coated PDMS was characterized using scanning electron microscope (SEM). The pattern samples without cells were dehydrated using serial alcohol from 30% to 100% for 15 minutes each, while the SiO$_2$ coated PDMS with hWJ-MSCs cells (10$^5$) grown on the surface for 48 hours were fixated using 2.5% (v/v) glutaraldehyde in 0.1M cacodylate buffer. Then, the samples were further dehydrated by adding HMDS (hexamethyldisilazane) – ethanol 100% (2:1) and HMDS 100% for 20 minutes each. Any remaining solution was evaporated inside a fume hood overnight. Once fully dried, the samples were coated with gold and observed under scanning electron microscope (SEM) (SU 3500; Hitachi; Center of Advanced Science Bandung Institute of Technology).

2.4 MTT Assay

The biocompatibility of SiO$_2$-coated PDMS with the hWJ-MSCs was tested using MTT cytotoxicity assay with two controls; (1) SiO$_2$ dispersed in medium and (2) without any addition (neither PDMS nor SiO$_2$ particles). hWJ-MSCs were cultured until it reached passage 4, then the cells were grown with standard growth medium on each surface (1 & 2), including the two controls. After 1, 3, 5, and 7 days, the remaining medium on each treatment was replaced with 10 µl MTT reagent in 100 µl growth medium and then incubated in the dark for 4 hours at 37°C incubator with 5% CO$_2$. The MTT reagent
discarded, 100 µl of DMSO were added on each well, and incubated at room temperature for 5 minutes to dissolve the formazan crystal. Absorbance of the solution was read using a microplate reader at 570 nm (n = 3).

3. Results and Discussion

3.1 PDMS Surface Morphology.
Scanning Electron Microscope imaging was used to observe PDMS surface morphology before and after coating with SiO2 particles, and MTT assay sample. In the Figure 2 it could be observed that the width, the gap, and the depth of PDMS was measured at around 1.2 mm, 600 µm, and 1 mm respectively. The surface morphology of the PDMS after coating process (Figure 2b) showed a compacted SiO2 layer, which was produced from stamping the PDMS into SiO2 powder (Figure 1f). For the MTT assay sample (Figure 2c), the PDMS was further cleaned so the area that covered by SiO2 was only the top of PDMS, which restrict the area that only cell can live.

![Figure 2 Surface morphology of PDMS. (a) Before coating, (b) After Coating, (c) MTT assay sample that show the cross section and the top view of the sample. W is width, D is depth and G is gap.](image)

3.2 Viability of hWJ-MSCs on SiO2-coated PDMS and SiO2 particles
hWJ-MSCs viability were evaluated by seeding the cells (10^5) on the surface of SiO2-coated PDMS (SiO2-PDMS), with the addition of two controls; cells grown on the surface of a well plate (WP) and on SiO2 particles dispersed on a well plate (SiO2-WP). The MTT readings were taken on day 1, 3, 5, and 7 post-seeding. Overall, the addition of SiO2 both in well plate and on PDMS surface showed biocompatibility with the cells for 7 days. Cells grown on SiO2-PDMS pattern had the highest viability in comparison to the two treatments (Figure 3). Cell viability on well plate showed viability decline starting on day 7, while on SiO2-PDMS and WP cell viability kept increasing until day 7. Based on this result, SiO2 coated PDMS was biocompatible substrate for hWJ-MSCs. Similar result was also reported, that SiO2 coated surface was able to support better cell viability for 14 days [6 7].
Figure 3. MTT assay result to analyse biocompatibility between the cells and SiO₂ coated PDMS (SiO₂-PDMS) for 1, 3, 5, and 7 days, using cells grown on well plate (WP) and SiO₂ particles (SiO₂-WP) only as control.

Figure 4. The morphology of hWJ-MSCs on SiO₂-coated pattern/PDMS at 21x, 100x, 300x, and 450x magnification. (a) Upper part of PDMS from Figure 4d with 100x magnification, (b) Lower part of PDMS from Figure 4d with 100x magnification, (c) 300x magnification of Figure 4a, (d) PDMS surface after 48 hours of cell attachment, (e) 450x magnification of Figure 4b. Right arrow (►) shows cells while left arrow (◄) shows SiO₂.
3.3 Morphology of hWJ-MSCs on SiO2-coated PDMS

SEM analysis was also used to observe the morphology of hWJ-MSC grown on the surface of SiO2 coated PDMS (Figure 4). Figure 4d shown a surface of PDMS after 48 hours of cell attachment, which the magnification of the upper part can be seen at Figure 4a, and the lower part at Figure 4b. Based on the SEM images, hWJ-MSCs were able to attach onto the SiO2 coated surface. The cells and the SiO2 can be differentiated by their morphologies which SiO2 has a morphology of irregular rock-like shape (shown by left arrow •) and cells appeared to have elongated, fibroblast like morphology, with few filopodia (Figure 4e and c) and spread evenly over the whole surface (shown by right arrow •). These results were also reported in a study by Tarpani et al [6] that MSC grown on SiO2 nanoparticles had elongated morphology, likely caused by higher hydrophilicity of surface [7, 8]

4. Conclusion

A parallel result was achieved between the effect on micropattern using SiO2 powder and synthetically produced using TEOS, showing a promising result that increase the viability of hWJ-MSCs cells. On this research, the SiO2-PDMS always shows the highest result of MTT assay up to 7 days, concluding a good biocompatibility and capacity to support cell proliferation between SiO2 powder with hWJ-MSCs.

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5. References

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