BIOCHIP JOURNAL, Vol. 2, No. 1, 39-43, March 2008

Effective Methods to Improve the Biocompatibility of Poly (dimethylsiloxane)

Joong Yull Park[1,†], Chang Mo Hwang[1,2,†] & Sang-Hoon Lee[1]

[1]Department of Biomedical Engineering, College of Medicine, Korea University, Anam-dong, Seongbuk-gu, Seoul 136-705, Republic of Korea
[2]Korea Artificial Organ Center, Korea University, Anam-dong, Seongbuk-gu, Seoul 136-705, Republic of Korea
[†]These authors contributed equally to this work.
Correspondence and requests for materials should be addressed to S.H. Lee (dbiomed@korea.ac.kr)

Accepted 16 February 2008

Abstract

Poly (dimethylsiloxane) (PDMS) has become one of the popular materials in the field of bio-related microfluidic systems, and has been used for various biological assays. While the material is known to be biocompatible in general, it also has been reported that the attachment and survival rate of cells are limited on a PDMS substrate. In this paper, a simple and relevant method to improve the biocompatibility of PDMS is proposed; the effects of various treatments using ethanol, water, and boiling-water were evaluated. The results show that the boiling-water treatment is the most appropriate, time saving, and simple method to improve the biocompatibility of PDMS.

Keywords: Biocompatibility, PDMS, Microfluidic chip, Fibroblast

Introduction

Since poly (dimethylsiloxane) (PDMS) was first proposed by early pioneers such as Whiteside[1] and Effenhauser[2], the material has become popular materials for building microfluidic devices. Many researchers have noted its advantageous characteristics such as optical transparency, stretchability, gas permeability, electrical insulation, low thermal conductivity, and low water permeability. In addition, it is relatively easy to fabricate cell-based micro systems using PDMS and soft lithography, and the material is known to be biocompatible[3-7]. This fact has resulted in enthusiastic studies on bio-MEMSs (bio-micro electro mechanical systems)[8].

Although PDMS is known to be biocompatible, it was also reported that PDMS is an inherent inhibitor for cell attachment[9]; cells were only attached on the patterning of cell adhesion factor in the PDMS device, and the attachment of the cells onto a bare PDMS surface was suppressed distinctly. Cell growth on PDMS having different ratios of base to curing agent was investigated, and meaningful differences were found[10]. Recently, the survival rate of mammalian neurons on a washed PDMS microdevice was examined using microfluidic devices having open and closed channels, and it was shown that extracting the PDMS improves the biocompatibility of microfluidic devices[7].

The goal of the work reported in this paper is to propose a practical, convenient and cost/load-effective treatment for PDMS modification. The effects of various treatments for PDMS using (1) ethanol, (2) deionized water (DI water), and (3) boiling water were evaluated and are discussed; improvement of the biocompatibility of PDMS for cells such as fibroblasts was demonstrated by using optical imaging and various cell-viability/functioning assays (Figure 1). The purposed method in this study can provide a simple and practical guideline for the treatment of fabricated PDMS device in order to improve their biocompatibility, and thus, to be used as a platform for a biological assay.

Results and Discussion

PDMS Membrane Fabrication and Contact Angle Measurement

The PDMS membranes were successfully fabricated (Figure 2); three membranes were used for each case listed in Table 1, and therefore, each datum presented in this paper corresponds to the average values of the three substrates. After the three treatments, the membranes were dried for 3 hours at 60˚C. Then, we measured the contact angle of the samples and found that there was no significant difference among the membranes (Figure 3). Only the Petri dish had a contact angle of 60˚, and this value can be judged to be hydrophilic; however, the other membranes had contact angles of about 115˚, which is clearly hydrophobic. This implies that the treatments did not alter

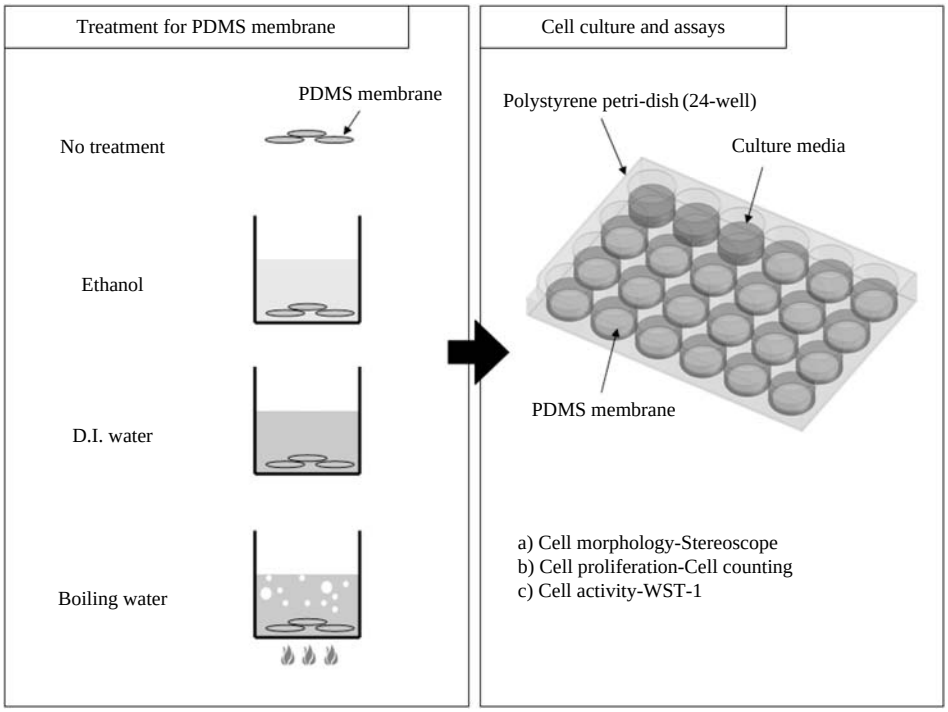

Figure 1. Illustration of treatments for the PDMS membranes and the experimental procedure.

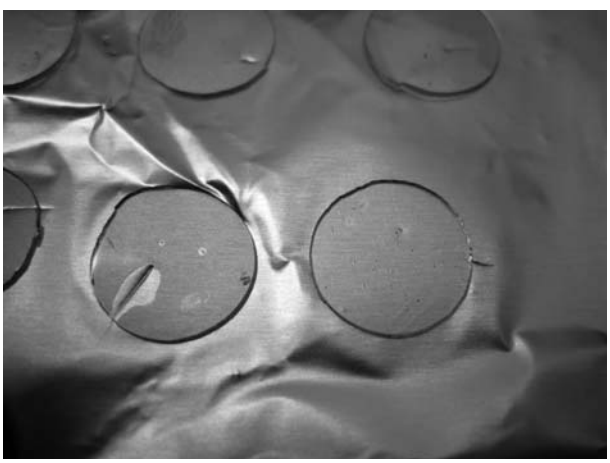

Figure 2. Fabricated PDMS membranes.

Table 1. Treatment type and time.

Treatment type	Treatment time
Petri-dish	–
No treatment	–
Ethanol treatment	12 h 24 h
Water treatment	12 h 24 h
Boiling-water treatment	5 min 60 min

the surface characteristics of the PDMS membrane. However, by the contact angle analysis, we found that the difference in hydrophilicity was not a factor that should be considered in this comparative study. Although, it is noticeable that the moderate hydrophilicity of the Petri dish (contact angle of 60˚) certainly gives it an advantage for cell cultures[11].

Morphology of the Cells and WST-1 Assay

After seeding, the L929 cells were allowed to attach onto the PDMS membrane for 4 hours. The cell attachment was observed every 2 hours on the first day. The attachments of the cells on the PDMS membranes were found to be weak when compared to the cells seeded on the Petri dish. One typical difference from a morphological view was that the cells on the PDMS membranes began to create clusters rather than spread (Figure 4a). Note that the cells kept their spherical shape when the outer circumstance was a harsh environment, while the cells on the normal culture dish developed healthy filopodia and spread well (Figure 4b). The overall status of the cell morphology was observed every 24 hours for 8 days. Table 2, though based on the subjective view of the authors, provides a good insight on the overall proliferation and morphology of the cells during the 8-day of culture period.

It is important to closely investigate the morpholo-

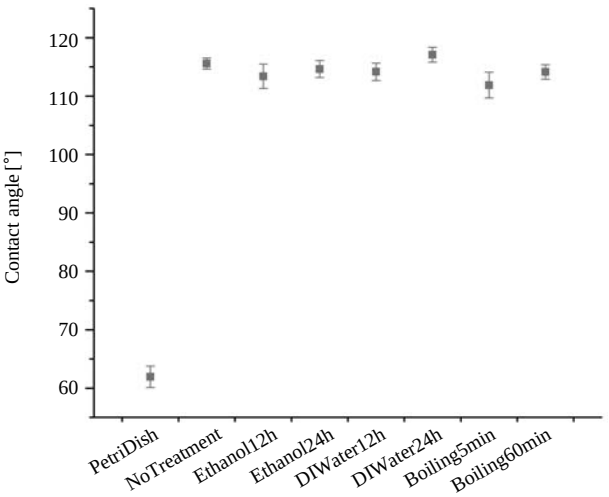

Figure 3. Measured contact angles on a PDMS membrane after each treatment.

Table 2. Overall morphological condition of the cells for each treatment.

Treatment type	Treatment time	Cell culture period			
		1-day	2-day	5-day	8-day
Petri-dish	–	++	++	+++	++++
No treatment	–	–	–	+	+
Ethanol treatment	12 h	+	+	+	++
	24 h	+	+	++	+
Water treatment	12 h	–	–	+	+
	24 h	+	+	+	+
Boiling -water treatment	5 min	–	+	+	++
	60 min	++	++	+++	++++

gical change of the cells in the different PDMS membranes as the examples in Figure 5 show well. The L929 cells cultured on all of the PDMS membranes, except for the boiling-water treatment for 60 min and Petri dish cases. A strong tendency to congregate together was found even an affluent number of cells presented.

Cell densities on the modified PDMS membranes were measured and compared (Figure 6); the cell densities are substantially different from one another. The cell number for the non-treated PDMS membrane was minimal, which implies that the PDMS itself is an inherent inhibitor for cell attachment[9]. All of the other treatments were shown to have either a small or large influence on cell proliferation, and the number of cells on the boiling-water treated PDMS was significantly higher than the other cases. We reasoned that boiling-water treatment seems to be

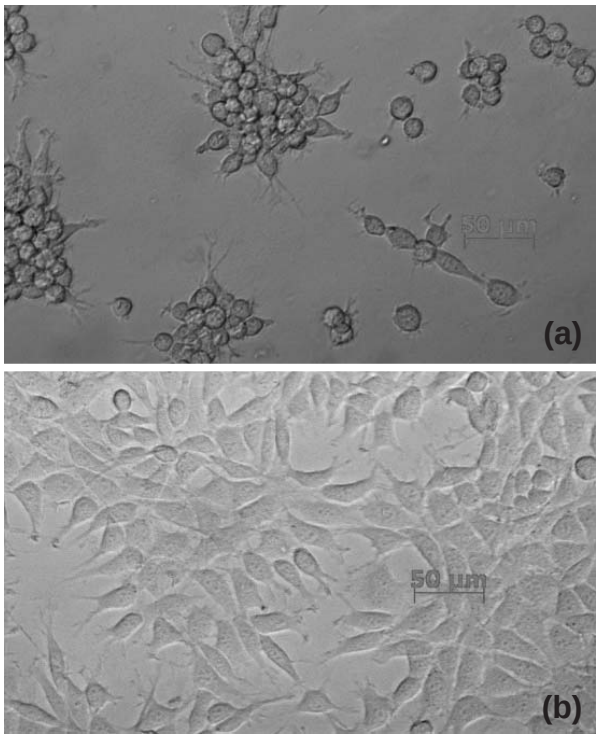

Figure 4. (a) Clustering cells were found on PDMS membranes, and in contrast, (b) the cells on the Petri dish spread well and are in healthy condition.

effective in the removal of harmful chemicals and uncross-linked agents in PDMS.

A WST-1 assay was conducted to evaluate the quantitative values of cell functioning and viability (Figure 6). The results imply that non-treated PDMS and ethanol-treated PDMS should be avoided. The most effective treatment is boiling-water treatment for 60 min. The 5 min-boiling treated PDMS membrane shows a comparatively high compatibility with the 60 min-boiling treated PDMS membranes. However, one should note that the morphological view of the cells in the 5 min-boiling treated PDMS is clearly different from that of the 60 min-boiling treated PDMS. Even though the cell viability and cell density were almost same in both cases, the cell conditions can be very different. This implies that the high temperature is an important factor for the water treatment.

Conclusions

For improvement of the biocompatibility of PDMS, the most appropriate treatment was found to be a boiling-water treatment because it requires neither a long time nor toxic chemicals, and it guarantees good

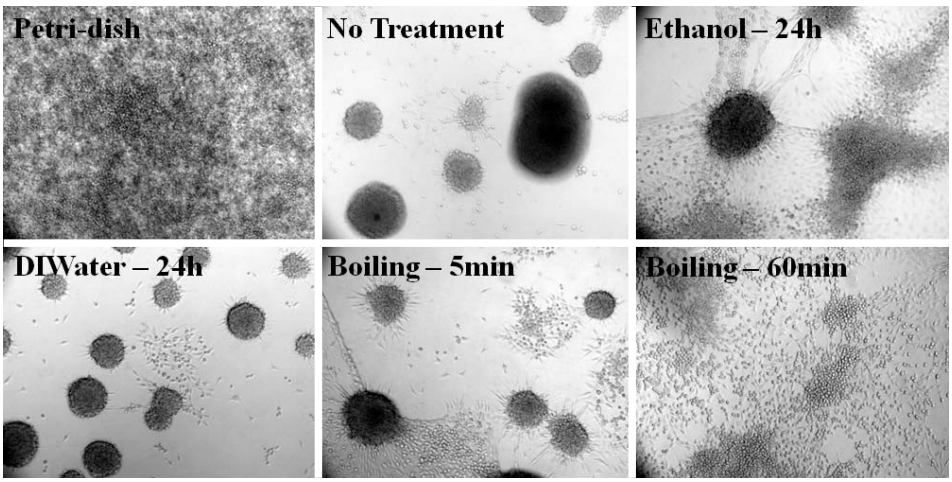

Figure 5. Bright field images of the morphology of the L929 cells on the modified PDMS membranes after an 8-day culture period.

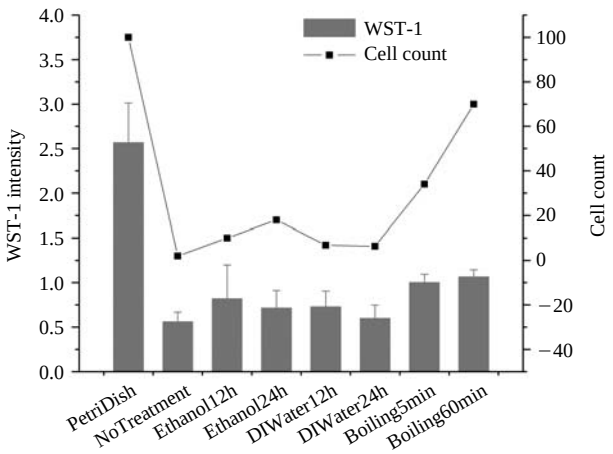

Figure 6. An L929 cell density measurement and WST-1 assay were conducted after an 8-day culture period on the modified PDMS membranes.

cell morphology and functioning. However, regarding only the metabolism itself, there is another option of using 24-hour dipping treatment of ethanol and DI water.

We proved that the PDMS is not cell-friendly though it is known to be biocompatible, and that a simple treatment using boiling water can improve its biocompatibility. In a practical point of view, the boiling water treatment can be conveniently used for improvement of the biocompatibility of cell-related PDMS devices.

Materials and Methods

Preparation of PDMS Membranes

Thin disc-like PDMS membranes (diameter: 15 mm, thickness: 1 mm) were prepared to culture the cells on them. A conventional protocol was used; a ratio of 10 : 1 for the PDMS: crosslinking agent was mixed by hand for 5 min and then degassed for 1 hour under a vacuum until all of the gas bubbles were removed. The PDMS was then poured onto a prepared flat glass substrate to achieve a thickness of 1 mm, and then cured at 80˚C for 3 hours and cooled down at room temperature for 24 hours. Finally, same sized PDMS membranes were made using a punch (diameter: 15 mm).

The experimental process used in this study is illustrated in Figure 1; three different treatments, dipping in (1) ethanol, (2) DI water, and (3) boiling water, were tested to evaluate the effects on the biocompatibility of PDMS, and these three treatments were also subcategorized by treatment time as listed in Table 1. For convenience, the three treatments are termed as ethanol treatment, water treatment and boiling-water treatment. A polystyrene Petri dish and non-treated PDMS were used as the control group. To avoid any unwanted side effects, no other treatment such as fibronectin coating was applied.

As one of preliminary evaluations of surface modification from the treatments, we measured the contact angles of each sample after each treatment. A goniometer (EasyDrop, KRÜSS GmbH, Germany) was used to measure the contact angels on the PDMS membrane substrates.

Cell Culture and Assays

Rat fibroblast cells (L929) were used in this experiment. The PDMS membranes were laid in each 24-well cell culture dish, and the cells were cultured on them. Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) with high glucose was used for a cell culture medium. For the homogeneous seeding of the

cells, the membranes were prewetted with the cell culture medium for 30 minutes. Then, the cells were seeded in each well with cell numbers of 2×10^3. Cell attachment and morphology were observed at 24-hour intervals. For the quantitative measurement of cell proliferation, a WST-1 cell proliferation assay kit (Roche Diagnostics GmbH, Germany) was used; WST-1 enables the measurement of the cell proliferation and cell viability with a colorimetric assay based on the cleavage of tetrazolium salts by mitochondrial dehydrogenase in the viable cells.

Acknowledgements

The authors are thankful for Jin Young Lee for her good assistance in the cell cultures. This study was supported by grants from KICOS (M 60601010002), and the Brain Korea 21 Project in 2008.

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