

BIOCOMPATIBILITY OF A PDMS-COATED MICRO-DEVICE: BLADDER VOLUME MONITORING SENSOR*

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Abstract We evaluated the biocompatibility of a dimethylpolysiloxane-coated micro-device which had been designed for monitoring real-time bladder volume in previous studies. The extract assay with dimethylpolysiloxane which had been used for coating the micro-device to measure the bladder volume was performed as an *in vitro* cytotoxicity test. For *in vivo* biocompatibility testing, the inflammatory responses around the implantation site of the micro-device in subcutaneous tissue of rat were assessed by light microscope with H&E stain and fluorescence microscope with ED1 stain and von Willebrand factor stain. The averages of cell viability in dimethylpolysiloxane group were 84.6% and 82.3% at 24 h and 72 h incubation, respectively. The qualitative evaluations with light and fluorescence microscope revealed that the inflammatory changes peaked during 2 weeks but almost disappeared at 4 weeks after implantation of devices. The quantitative evaluations for granulation layer formation and neovascularization showed that the thickness of the layer in dimethylpolysiloxane group peaked during 2 weeks but it came to be stabilized at 4 weeks as thin as at 2 weeks in control group, and the frequency of neovascularization was higher in dimethylpolysiloxane group than in control group but it was not increased with time. The dimethylpolysiloxane-coated micro-device is thought to be a reliable bio-medical device.

Keywords: Dimethylpolysiloxane; Devices; Biocompatibility testing.

INTRODUCTION

Real-time monitoring of volume status in some chronic diseases is important for patients to avoid complications related to high pressure in limited space, and trials to solve such problems are making steady progress in various clinical fields with the development of implantable micro-devices by application of microelectrical mechanical systems (MEMS) technology^[1]. Important concepts for implantable micro-devices include micro-invasiveness and biocompatibility. Recently, with much improvement in the technical aspects of such devices, a micro-device has been reported in the urological field that can work in the urinary bladder to enhance the quality of life of neurogenic bladder patients by announcing the optimal timing for urination and preventing complications induced by overdistension of a dysfunctional neurogenic bladder^[2]. Recently, authors

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have developed a dimethylpolysiloxane (PDMS)-coated micro-device that can be implanted in the external walls of the urinary bladder to monitor real-time bladder volume *in vivo*^[3]. These studies have achieved marvelous results, but with regard to biocompatibility, which is one of the major concerns for a medical device, the studies were lacking.

Therefore, an in-depth discussion of the biocompatibility of implantable bladder-volume-monitoring devices is necessary. In our previous study, we used PDMS as a coating material for the sensor. In this study, we evaluated the cytotoxicity of PDMS *in vitro* and evaluated tissue responses after implantation of PDMS-coated bladder volume sensors into dorsal subcutaneous tissues of rats. Our results will help to provide basic data for assessment of the safety of urinary bladder-volume-monitoring sensors for future clinical trials.

EXPERIMENTAL

All animal experiments were conducted following the guidelines and regulations pertinent to animal experiments of the Institutional Animal Care and Use Committee, Catholic University Medical College (CUMC-2010-0066-01).

Preparation of Micro-device with PDMS Coating

The Pyrex glass was washed with acetone and poly-silicon was deposited on both sides at a thickness of 300 nm. With the use of a spin coater, photoresist was done followed by soft baking. Through the cracks in the honeycomb shape on the mask, the substrate was exposed to ultraviolet light and the membrane of poly-silicon was partially removed. Then the etching process was done with hydrofluoric acid solution to 100 μm in depth. The photoresist was then removed by acetone washing and the remnant poly-silicon was also removed by reactive ion etching. Au was deposited on the working side at a thickness of 100 nm. To complete the MEMS process, the sample was dipped into PDMS mixture (Sylgard 184, Dow Corning, silicone elastomer:curing agent = 10:2), and the samples coated with PDMS mixture were cured in an oven at 80°C for 2 h.

In vitro Biocompatibility Study with PDMS

The cytotoxicity test of the prepared packaging material was determined by cell counting with trypan blue. In the dynamic condition, extract of the test sample, PDMS, was made by immersing one micro-device sample into Dulbecco's modified eagle medium (DMEM) containing 100 units/mL of penicillin, 100 g/mL of streptomycin and 10% FBS in an incubator at 37°C in a humidified 5% CO₂ atmosphere for 24 h. First, NIH3T3 fibroblasts (1×10^6 /well) were plated into 6-well plates and incubated for 24 h. Then, the medium was aspirated and rinsed twice with PBS. The extract of the test sample was added to each well, and the plates were then incubated for 24 h and 72 h. The cells detached by trypsin/EDTA were stained with trypan blue (500 μL 4%, Gibco). The living cells were subsequently counted in a hemocytometer.

In vivo Biocompatibility Study with PDMS-coated Micro-device

Implantation of the micro-device in the dorsal subcutaneous tissue of rats

Twenty-four male Sprague-Dawley rats weighing 250–300 g, housed in a temperature- and humidity-controlled environment on a 12/12-h light-dark cycle, were randomly assigned to two equal groups: *i.e.*, group 1 (controls, sham operation), and group 2 (micro-device implantation). They were fed standard rat food and water, freely available, and acclimatized to the laboratory environment for at least 1 week before surgery. Then, all rats were anesthetized by the intramuscular injection of ketamine (Ketamine; Yoochan Yanghang, Seoul, Korea, 75 mg/kg) and xylazine (Rompun, Bayer Korea, Korea, 15 mg/kg) for surgical manipulation. In the experimental group (group 2), methodologically by ISO 10993-6, the back area of each rat was sterilized with 10% povidone-iodine solution and incised by sterile techniques, the subcutaneous tissues were exposed, and a micro-device [2298 μm (l) $\times$ 3200 μm (w) $\times$ 500 μm (h)] was placed in direct contact with the subcutaneous tissues. In the control group (group 1), sham operations were performed for 5 min by use of toothless forceps. The 12 rats in each study group were randomly divided into four subgroups that were sacrificed at 7, 14, 21, and 28 days after surgery.

Assessment of tissue granulation

Tissues around the micro-device were collected and fixed in 10% neutral formalin for 1 day. For the preparation of the fixed tissues as samples for light microscopy, the tissues were dehydrated with alcohol, embedded in paraffin, sectioned in 5 µm widths by use of a microtome, and stained with hematoxylin-eosin (H&E). A pathologist unaware of the experimental protocols examined the tissues at the implantation site by use of a light microscope and measured the maximum thickness of the granulation layer adjacent to implantation site.

Assessment of neovascularization

Sectioned tissues were immobilized on slides, immersed in 100% xylene for 3 min each, subsequently immersed in ethanol from 100% to 60% sequentially for 2 min each, and then washed for 5 min. The slides were added to 0.01% sodium citrate (pH 6), boiled in a microwave oven, and cooled at room temperature for 20 min. To inhibit nonspecific reactions during staining, the samples were reacted with a blocking solution (1.5% normal goat serum, 1.5% normal horse serum, 1% BSA, 0.1% Triton X-100 in PBS) at room temperature for 1 h and then reacted with the first antibody (mouse monoclonal von Willebrand factor; Abcam, UK) at room temperature for 2 h. The samples were then washed in PBS 3 times and reacted with the secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG; Invitrogen, USA) diluted with the blocking solution to 1:500 at room temperature for 1 h. The samples were then washed with PBS 3 times and mounted with DAPI, and images were obtained by use of a fluorescence microscope.

The micro-vessel density (MVD) in one section from each specimen was assessed by fluorescence microscope. In each case, the most intensely stained vascular area around the implanted bio-device was identified at X40 and the vessels in three separate high-power fields (X400) within this area were counted. Therefore, the mean of nine sample counts in one subgroup was calculated as the MVD at each week.

Assessment of inflammatory reaction

Sectioned tissues were immobilized on slides, washed with 100% xylene for 3 min each, immersed in ethanol from 100% to 60% sequentially for 2 min each, and then washed for 5 min. The slides were added to 0.01% sodium citrate (pH 6), boiled in a microwave oven, and cooled for 20 min at room temperature. To inhibit nonspecific reactions during staining, the samples were reacted with a blocking solution (1.5% normal goat serum, 1.5% normal horse serum, 1% BSA, 0.1% Triton X-100 in PBS) at room temperature for 1 h and reacted with the primary antibody ED1 (mouse Anti rat-CD68, Serotec, UK) at room temperature for 2 h. The samples were then washed with PBS 3 times and reacted at room temperature for 1 h with the secondary antibody (Alexa Fluor 568 goat anti-rabbit IgG; Invitrogen, USA) diluted to 1:500 with the blocking solution. The samples were then washed with PBS 3 times and mounted with DAPI, and images were obtained by use of a fluorescence microscope.

Statistical analysis

The statistical analyses were done by using SPSS® software version 15.0 (SPSS Inc, Chicago, IL, USA). The results were assessed by using mean values. The groups were compared at 7, 14, 21, and 28 days and within-group comparisons were made by using the Mann-Whitney U-test and the Kruskal-Wallis H-test.

RESULTS

In vitro Biocompatibility

The cell count in each well before and after treatment with the extract of the prepared packaging material is presented in Table 1. The average cell viabilities in the control group were 92.7% and 92.3% at 24 h and 72 h incubation respectively, which were higher than those in the experimental group (84.6% and 82.3%) by 8.1% and 10.0% ($p < 0.05$ by Mann-Whitney U-test). However, within experimental group, there was no statistical difference of cell viability by time ($p = 0.24$ by Mann-Whitney U-test).

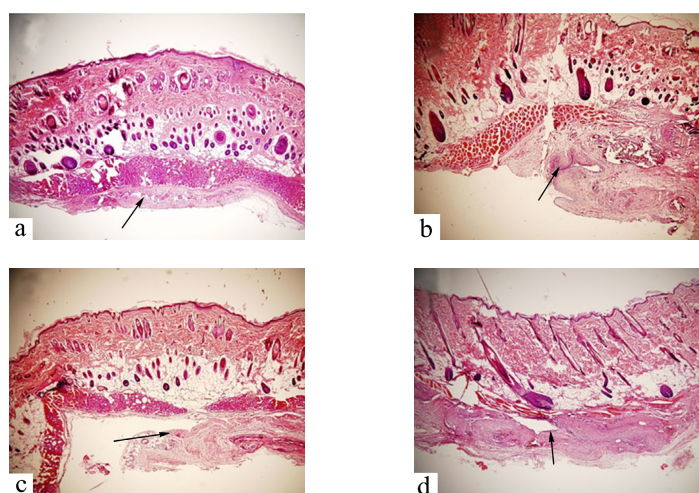
Table 1. *In vitro* cytotoxicity testing by the extract assay

	24 hours*	Cell viability (%)	72 hours*	Cell viability (%)
Control	2.29/2.42	94.6	2.31/2.46	93.9
	2.12/2.32	91.2	2.67/2.88	92.8
	2.69/2.92	92.2	2.09/2.24	91.9
	2.36/2.58	91.5	2.85/3.11	91.7
	2.06/2.20	93.6	2.30/2.51	91.8
	2.88/3.10	92.8	1.82/1.99	91.4
PDMS	2.43/2.81	86.6	1.85/2.33	79.5
	2.08/2.44	85.2	1.96/2.38	82.3
	1.81/2.16	83.8	2.41/2.95	81.7
	2.48/3.02	82.1	2.02/2.42	83.5
	2.78/3.15	88.2	1.98/2.40	82.4
	1.68/2.06	81.6	2.21/2.63	84.1

* Viable cells ($\times 10^6/\text{mL}$)/Initial total cells ($\times 10^6/\text{mL}$)

***In vivo* Biocompatibility**

Two rats in the *in vivo* biocompatibility study died. One was in the control group and died 2 days after surgery. The other was in the experimental group and died 22 days after surgery. The rats that died had no gross abnormal findings. When the other rats were sacrificed for *in vivo* evaluation, there were no definite signs of edema, adhesion, or redness, except for tiny fibrotic changes adjacent to the surface of the micro-device (Fig. 1).

**Fig. 1** Gross finding after 4 weeks of implantation**Fig. 2** Microscopic findings with H&E stain (a) control group at 2 weeks; (b), (c) and (d) experimental groups at 2 weeks, 3 weeks and 4 weeks, respectively

When we used light microscopy with H&E staining to observe the inflammatory response at a glance, we found thin granulation layers at 1 week and 2 weeks that disappeared after 3 weeks in the control group. In the experimental group, however, the capsules were over 3 times as thick as those in the sham operation group and contained more abundant immune-reactive cells. Nonetheless, the thickness of layers tended to decrease after 3 weeks and at 4 weeks it became as thin as at 2 weeks in control group. The mean granulation layer thickness was about 14 μm and 46 μm in the control and experimental groups, respectively, at 2 weeks when the maximal thickness was seen in each group. We could find neither necrotic area nor foreign debris (Figs. 2 and 3).

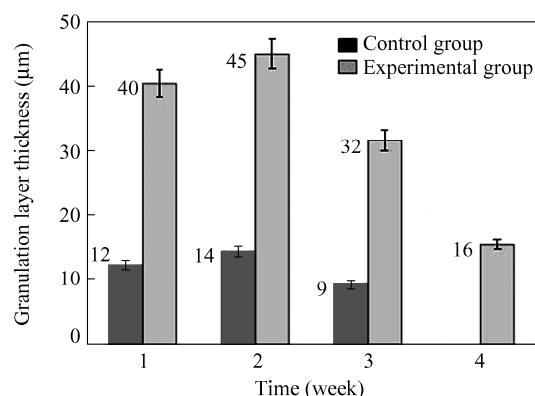


Fig. 3 The granulation thickness around the PDMS-coated micro-device implantation site

With respect to inflammatory reaction, we found that macrophage infiltration peaked at 1 week and the cells had nearly disappeared at 4 weeks as if no surgical procedure had been done (Fig. 4). In the control group, however, macrophages were nearly invisible even at 2 weeks and were as scant as at 4 weeks in the experimental group.

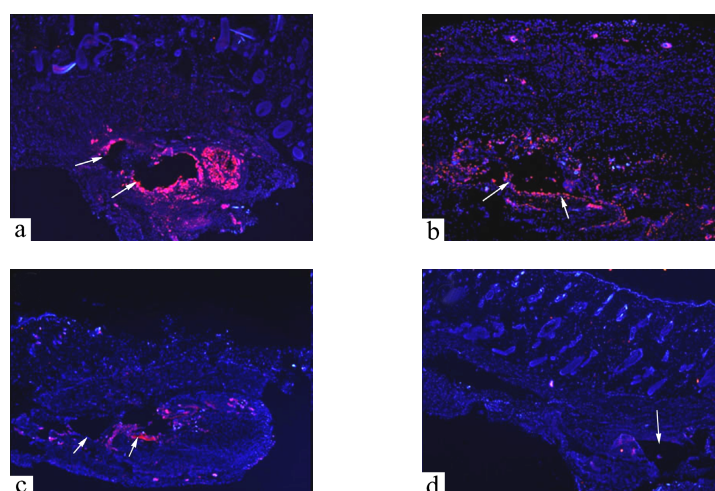


Fig. 4 Macrophage infiltration as shown by fluorescence microscope after implantation of a PDMS-coated micro-device (a) 1 week, (b) 2 weeks, (c) 3 weeks and (d) 4 weeks. White arrows: implantation site of micro-device; Dark: cell nucleus (DAPI), Bright: macrophage, ED1 staining, $\times 40$

Neovascularization around the implantation site was observed with von Willebrand factor stain mounted with DAPI by use of a fluorescence microscope. MVD was about 2.5 times higher in the experimental group than in the control group during the whole experimental period (Fig. 5). However, in each group, MVD showed

no statistical difference (Table 2). As previously described, 3 samples were collected to calculate MVD in one rat; MVD was calculated with 9 samples in each subgroup. Before 1 week, however, one rat in the control group had died and the mean value at 1 week was thus calculated with 6 samples. This also happened at 4 weeks in the experimental group.

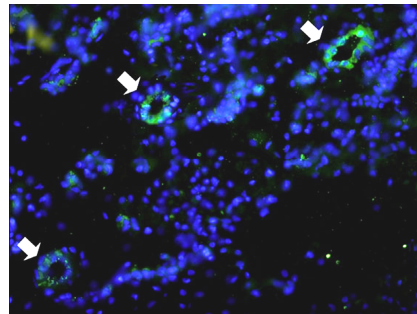


Fig. 5 Staining for von Willebrand Factor
Arrows: green-blood vessel, blue-cell nucleus (DAPI),
von Willebrand Factor, $\times 400$

Table 2. Micro-vessel density

MVD (n/HPF)	Control	PDMS
1 week	1.50 ± 0.55	3.78 ± 0.67
2 weeks	1.44 ± 0.53	3.89 ± 0.78
3 weeks	1.33 ± 0.50	3.56 ± 0.73
4 weeks	1.33 ± 0.50	3.67 ± 0.52
<i>p</i> -value	0.88	0.75

The data are expressed as mean $\pm$ standard deviation of the mean;
Comparison within each group was done with the Kruskal-Wallis H-test.

DISCUSSION

Usually, neurogenic bladder patients cannot recognize or have a decreased sense of the distension over their normal bladder capacity. As a result, normal voiding does not occur and, moreover, repetition of inappropriate voiding causes deterioration of renal function. Therefore, warning of distension of the urinary bladder and proper control of the voiding time can reduce morbidity in these patients. In this sense, a technical trial to monitor real-time bladder volume was proposed by implantation of a bio-medical device in the urinary bladder^[4]. Recently, we reported the attachment of real-time, implantable bladder volume sensors to both lateral sides of the outside wall of the bladder by application of MEMS technology^[3].

A device to be implanted in the human body must be 'safe' for the patient, *i.e.*, it must be biologically compatible with tissues^[5]. However, evaluations of safety have rarely performed in the urological field, especially with implantable bio-medical devices. PDMS is a well-known bio-material for which biocompatibility tests have been described in many publications^[6]. Nevertheless, research on PDMS as a coating material for an implantable micro-device is not yet sufficient. PDMS is suitable as a coating material for electronic sensors because of its tension stiffening characteristics either at room temperature or at high temperatures. In the case of the Sylgard 184 kit, a commercialized raw material for PDMS, the vinyl group contained in a prepolymer together with silicon hybrid contained in the curing agent forms a crosslink reaction such as $\text{Si}-\text{CH}_2-\text{CH}_2-\text{Si}$ with the help of an organometallic catalyst. In this process, raising the proportion of curing agent from 10% to 20% can increase the tensile strength and improve Young's modulus^[7]. To fabricate the micro-device, PDMS with 20% curing agent was used as the coating material. The extract sample test was done as described in the ISO 10993-5 to evaluate the cytotoxicity of PDMS. In our study, the averages of cell viabilities in the experimental group were 84.6% and 82.3% at 24 h and 72 h incubation, respectively which were lower than

those in the control group (92.7% and 92.3%) statistically. However, within experimental group, there was no statistical difference of cell viability by time. According to ISO 10993-5, with quantitative evaluation, a reduction of cell viability by more than 30% is considered a cytotoxic effect. Bélanger *et al.* described in their work that cell viability over than 80% was favorable and between 60%–80% was slight reduction^[6]. Accordingly, PDMS extracted from the micro-device in this study is considered to be a safe material. In general, early after implantation of bio-medical device, protein adsorption would occur on the surface material of the device. Then, a provisional matrix or avascular capsule would be formed in sequence. Whereas the resulting foreign body reaction typically leads to the formation of an avascular capsule that is beneficial in that it isolates the foreign body from the surrounding tissue, the inflammatory response and the modulation of the inflammatory response by the bio-material can contribute to the development of device-related complications such as bio-material-centered infection, implant degradation or loosening, thrombosis, contraction of the fibrous capsule, and so on^[8, 9].

The tissue inflammatory response can be assessed by light microscopy with H&E staining. Dalu *et al.* reported the difference in inflammatory response between the sexes in mice where small pieces of a smooth PDMS was implanted in the interscapular region and evaluations, such as measurement of granulation layer thickness around the implantation site, were made primarily on the central area of the implant, away from the edges and then on relative numbers of inflammatory cells around the implantation site^[10]. We also assessed the thickness of the avascular layer or granulation layer at the implantation site. We found that the maximum thickness of the capsular layer was seen at 2 weeks in both groups, and in the experimental group, the layer was much thicker than in the control group. The density of inflammatory cells in the layer was also highest at 2 weeks. However, the thickness of the layer and its density decreased with time, which suggests a stabilization of the layer and a dwindling of the immune response. To observe inflammatory cell infiltration, we used immunohistological staining with ED1 antibody to see the existence of macrophages around the implantation site. Bio-material-mediated inflammatory response is modulated by histamine-mediated macrophage recruitment and adhesion to the implant surface^[11]. Importance of the macrophage in contribution to acute and chronic inflammatory reaction is well known^[9, 12]. For this reason, many researchers use immunohistological stains such as MAC387, ED1, or CD4 as macrophage markers^[13, 14]. Here, we found that the brisk reactions of macrophages were observed at 1 week and were gradually reduced. At 4 weeks, the macrophages nearly disappeared. As a result, the degree of inflammatory response was not maintained throughout the experiment, but rather ceased with time. Quantitative assessments with inflammatory cell counting can help studies more objective, especially when qualitative assessments get equivocal results. One of limitations in our study is the lack of such quantitative assessment for cell counting, although in this study, the aspect of macrophage infiltration in each week was quite distinguishable from each other.

Angiogenesis, which is defined as the formation of new blood vessels from existing vasculature, is important during development. In adults, the process is limited to the female reproductive system and to tissue repair and pathological conditions. In the normal tissue-healing process, neovascularization peaks by day 5^[15]. There have been many reports in which the inflammatory reaction was assessed by observation of neovascularization^[16]. In our study, we described neovascularization as MVD, which was higher in the experimental group than in the control group by approximately 2.5 times. Interestingly, MVD was not significantly different within each group throughout the experiment. This suggests that the inflammatory response was activated only during the early few days after micro-device implantation.

CONCLUSIONS

In this study, PDMS, which was used as a coating material for a bladder volume sensor, was biocompatible according to the *in vitro* extract assay. In the *in vivo* study, formation of granulation capsules with a tendency for a decrease in macrophage infiltration revealed that the tissue around the implantation site was stabilized with time, which could explain why MVD was fixed after 1 week in each group. So, despite the inflammatory response seen during 2 weeks in the experimental group, the PDMS-coated micro-device must be a reliable bio-medical device. We expect that biocompatibility studies such as ours on PDMS-coated micro-devices will be

novel baseline research for the development of ambulatory urodynamic devices in the future.

More studies will be needed to evaluate the inflammatory response with other immune-reactive cells, including fibroblasts, lymphocytes, and so on. Such research would be more worthy if long-term studies could be performed.

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