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ORIGINAL ARTICLE

Real-time bladder volume monitoring by the application of a new implantable bladder volume sensor for a small animal model

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Abstract Although real-time monitoring of bladder volume together with intravesical pressure can provide more information for understanding the functional changes of the urinary bladder, it still entails difficulties in the accurate prediction of real-time bladder volume in urodynamic studies with small animal models. We studied a new implantable bladder volume monitoring device with eight rats. During cystometry, microelectrodes prepared by the microelectromechanical systems process were placed symmetrically on both lateral walls of the bladder, and the expanded bladder volume was calculated. Immunohistological study was done after 1 week and after 4 weeks to evaluate the biocompatibility of the microelectrode. From the point that infused saline volume into the bladder was higher than 0.6 mL, estimated bladder volume was statistically correlated with the volume of saline injected ($p < 0.01$). Additionally, the microelectromechanical system microelectrodes used in this study showed reliable biocompatibility. Therefore, the device can be used to evaluate changes in bladder volume in studies with small animals, and it may help to provide more information about functional changes in the bladder in laboratory studies. Furthermore, owing to its biocompatibility, the device could be chronically implanted in conscious ambulating animals, thus allowing a novel longitudinal study to be performed for a specific purpose.
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Introduction

When urodynamic studies are conducted in small animal models such as rats in a laboratory, intravesical pressure is commonly measured by transurethral catheterization or suprapubic puncture [1]. Such experiments usually require cumbersome processes to accurately predict changes in bladder volume, and there are some limitations for understanding the relationship of intravesical pressure with volume changes in real time.

Until now, several *in vivo* studies have been conducted on the measurement of bladder volume in real time, and the results are very encouraging. These pioneering research studies led to the development of new kinds of equipment for monitoring bladder volume in neurogenic bladder patients [2,3]. However, because of the sizes of the bladder volume monitoring devices and their sensitivities, such devices have limitations for application to small animal models.

Recently, equipment applying the microelectromechanical systems (MEMS) process has been studied in biomedical research and has been used for both clinical diagnosis and treatment [4—7]. Features of bio-MEMS technology include the use of biocompatible materials, micro-miniaturization, and creating implantable biodevices. In the present study, we measured bladder volume with a mini *in vivo* bladder volume sensor produced by MEMS engineering to evaluate the usefulness of bladder volumetry for a small animal in an experimental setting and to study the possibility of using a chronically implanted device.

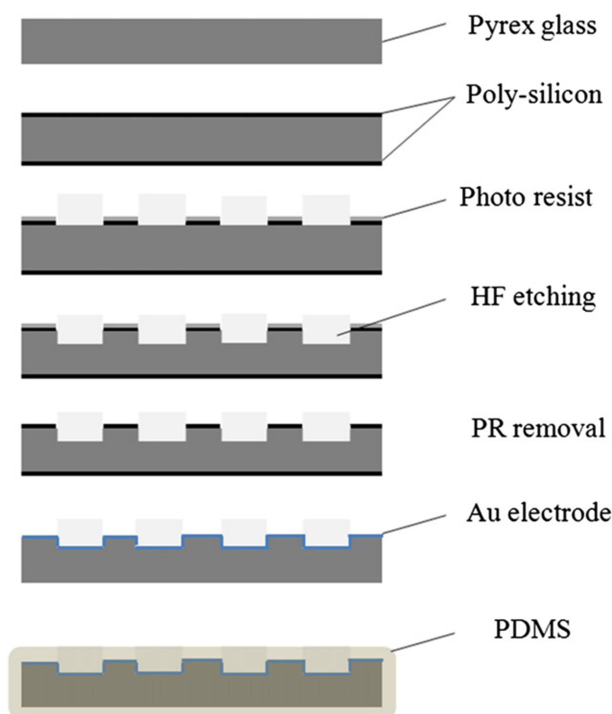

Figure 1. Preparation of the electrode by the microelectromechanical system process. Photolithography was done on Pyrex glass deposited with polysilicon. With an etching process, gold was deposited and finally PDMS packing was done. PDMS = polydimethylsiloxane.

Materials and methods

The research was conducted in accordance with the guide for the care and use of laboratory animals of the National Institutes of Health and was approved by the Catholic University Animal Ethics Committee (Catholic University Medical College (CUMC)-2010-0066-01).

Preparation of the electrodes by the MEMS process

After the Pyrex glass was washed with acetone, polysilicon was deposited on both sides to a thickness of 300 nm (Fig. 1). With a spin coater, photoresist was done followed by soft baking. Through the cracks of the honeycomb shape on the mask, the substrate was exposed to ultraviolet light, and the membrane of polysilicon was partially removed. Then with a hydrofluoric acid solution, an etching process was done to 100 μm in depth. The photoresist was then removed by acetone washing, and the remnant polysilicon was also removed by reactive ion etching. Gold was deposited on the working side at a thickness of 100 nm. After coating with polydimethylsiloxane (PDMS), the MEMS process was completed. At that time, the sample was dipped into PDMS mixture (Sylgard 184, Dow Corning; silicone elastomer:curing agent = 10:1), and the samples coated with PDMS mixture were cured in an oven at 80°C for 2 hours.

Experimental methods

As experimental models, eight Sprague-Dawley male rats weighing 250—300 g were used. Anesthesia was performed by the method of intraperitoneal injection of xylazine (Rompun; Bayer Korea, Korea, 15 mg/kg) and ketamine (Ketamine; Yoohan Yanghang, Seoul, Korea, 75 mg/kg). A vertical incision was made in the lower abdomen with the rat in the supine position, the urinary bladder was exposed, the bladder dome was punctured with a 25-gauge needle, and the tip was placed inside the bladder. Subsequently, using a three-way tube, room temperature saline was infused into the bladder at 0.1 mL/min with a KD Scientific syringe pump (KD Scientific Inc., Holliston, MA, USA), and the intravesical pressure was checked with a pressure transducer (Research Grade Blood Pressure Transducer; Harvard Apparatus, Holliston, MA, USA). Simultaneously, microelectrodes prepared by the MEMS process were affixed with 6-0 black silk externally on both lateral walls of the bladder symmetrically (Fig. 2). To minimize the size of the sensor, each electrode was designed as a honeycomb type in a rectangular shape, 2,298 μm (l) × 3,200 μm (w) × 500 μm (h) in size (Fig. 3). The distance (d) between the electrodes was converted from capacitance measured by the Locus Control Region meter (Agilent, CA, USA), which was externally connected to both electrodes during the infusion of saline into the urinary bladder. Assuming the filled bladder shape was a sphere, the bladder volume was estimated by applying the sphere volume formula ($1/6\pi d^3$) to simplify the process [2]. Concurrently, bladder pressure was recorded in the polygraph (Grass 7D; Grass Inst. Co., Quincy, MA, USA) through a transducer.

In each rat, from the time of the infusion of 0.2-mL saline into the bladder to the leak point from the filled

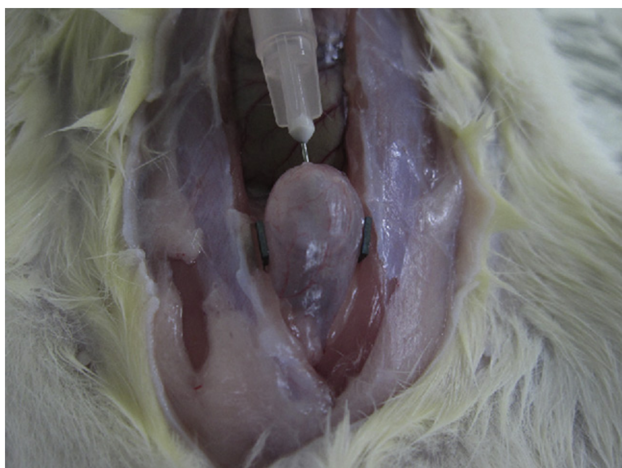

Figure 2. Photograph showing the microelectrodes affixed to both lateral walls of the urinary bladder.

bladder, the capacitance measured by the electrodes every 2 minutes was converted into distance, the volume was estimated, and the average value was obtained. After the experiment, the abdominal wound of each rat was closed, leaving each electrode affixed onto the external walls of the bladder.

Statistical evaluation

The estimated volume and the volume of saline infused at each interval were compared by using the Mann-Whitney *U* test. The correlation between the volume of saline infused and the volume estimated by the electrodes during the entire interval was assessed by regression analysis and repeated-measures analysis of variance. Statistical analysis was performed by using SPSS 12.0 (SPSS Inc., Chicago, IL, USA), and p values of <0.05 were considered statistically significant.

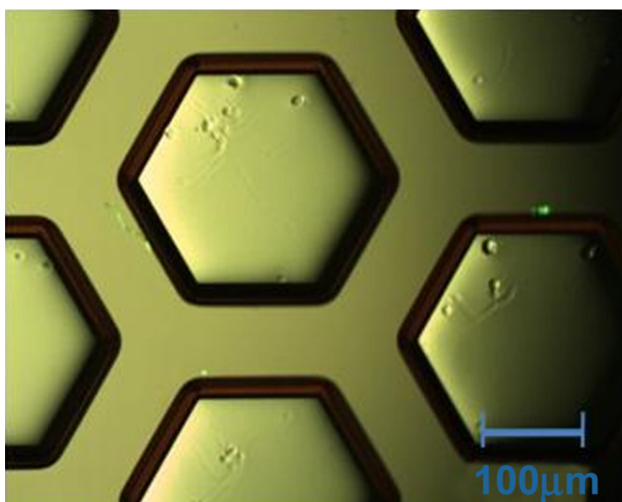

Figure 3. Photograph showing the surface structure of the microelectromechanical system electrode.

Evaluating biocompatibility

After 1 week and 4 weeks, the rats were sacrificed. Using ED1 (mouse antirat-CD68, Serotec, UK) staining of the tissue adjacent to the device, a fluorescence microscope was used to look for tissue immune responses to the MEMS electrodes packed with PDMS.

Results

At each 2-minute interval, we compared the volume of saline infused into the urinary bladder with bladder volume estimated by the MEMS electrode, ending at the point of saline leak from the bladder. Among all leak points (2.4—2.8 mL) of eight rats, the minimum was observed at 2.4 mL; therefore, we gathered the data from 0.2 mL to 2.4 mL for each rat.

From the point at which the infused saline volume was 0.6 mL, the volume of saline injected into the bladder and the bladder volume measured through the MEMS electrodes did not show a significant difference (Mann-Whitney *U* test $p > 0.05$, repeated-measures analysis of variance $p > 0.05$, and regression analysis $p < 0.05$), although there was a very slight tendency for overestimation by the electrode from the point at which the infused saline volume was 1.8 mL. On the other hand, when the volume of saline injected into the bladder was less than 0.6 mL, the bladder volume estimated by the electrodes was shown to be higher than the infused saline volume (Mann-Whitney *U* test $p < 0.05$) (Table 1).

The mean time for each experiment (surgical procedure plus cystometric evaluation) was 42 minutes. One rat died after the experiment.

With regard to biocompatibility, the fluorescence microscope images showed that macrophage accumulation was seen at 1 week but had almost disappeared at 4 weeks, thus showing reliable biocompatibility (Fig. 4).

Discussion

Information about voiding function can be obtained and used through urodynamic studies conducted in animal models. Hence, when urodynamic studies are performed in animal models, the accuracy of the equipment used and its usefulness are very important. Furthermore, improvements in the experimental equipment not only provide the convenience of supplying data in laboratories but also help the equipment itself exert an absolute role in clinical application. In the past decades, to enhance the accuracy of urodynamic testing in animal models and the convenience of conducting experiments, investigators have continually improved the equipment used. Nevertheless, monitoring the changes in bladder volume and postvoid residual urine volume is still difficult when small animals are used. Recently, such changes could be monitored appropriately by the use of a metabolic cage. With a small animal model, once it urinates, the excreted urine can be collected by a metabolic cage, and bladder capacity can be estimated by adding residual urine volume withdrawn from the catheter placed into the bladder [8]. It is true that such methods are very accurate owing to recent technical

Table 1 Estimated bladder volume by microelectromechanical system electrodes

Infused volume (mL)	Mean capacitance (pF)	Mean distance (mm)	Mean estimated volume (mL)[a]
0.2	5.13 ± 0.39	0.98 ± 0.09	0.49 ± 0.15[b]
0.4	4.88 ± 0.31	1.10 ± 0.07	0.67 ± 0.14[c]
0.6	4.86 ± 0.26	1.11 ± 0.06	0.72 ± 0.12[d]
0.8	4.72 ± 0.13	1.17 ± 0.03	0.83 ± 0.07
1.0	4.55 ± 0.09	1.24 ± 0.02	1.01 ± 0.06
1.2	4.41 ± 0.10	1.31 ± 0.02	1.19 ± 0.08
1.4	4.25 ± 0.13	1.38 ± 0.03	1.38 ± 0.09
1.6	4.04 ± 0.10	1.48 ± 0.02	1.68 ± 0.09
1.8	3.91 ± 0.09	1.54 ± 0.02	1.91 ± 0.06
2.0	3.80 ± 0.08	1.59 ± 0.02	2.09 ± 0.08
2.2	3.69 ± 0.04	1.64 ± 0.01	2.31 ± 0.08
2.4	3.57 ± 0.12	1.69 ± 0.03	2.53 ± 0.12

[a] $p < 0.05$ (regression analysis) versus infused volume through the entire interval and $p > 0.05$ (repeated-measures analysis of variance) versus infused volume from the point that volume is higher than 0.6 mL.
[b,c,d] $p < 0.05$ (Mann-Whitney *U* test) versus infused volume at each interval.
The data are expressed as mean ± standard error of the mean.

improvements. However, they need somewhat complex procedures or have shortcomings in that the monitoring of real-time changes in bladder volume concomitant with bladder pressure cannot be performed.

For the purpose of clinical use, several studies of *in vivo* types of devices for monitoring bladder volume have been conducted. *In vivo* devices have the advantage of being able to monitor real-time bladder volume. One study explained the usefulness of monitoring changes in bladder volume through the comparison of *in vivo* ultrasonography with external bladder ultrasonography [2]. The ultrasonography equipment used by those investigators was significant when the solution within the bladder was more than 50 mL during a urodynamic study; therefore, the usefulness of such equipment may be low in an experiment with small animal models. Another study reported an attempt to measure bladder volume by use of permanent magnetics. However, because of the size of the equipment, it was not readily prepared and was difficult to use in basic research with small animal models [3].

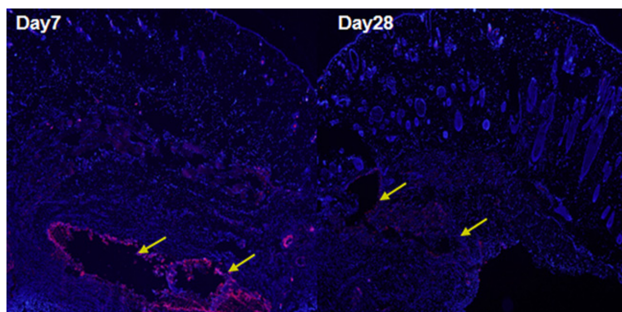

Figure 4. ED1 immunofluorescence staining (40×) of tissue adjacent to the microelectromechanical system electrode, which was packed with polydimethylsiloxane. Arrows: implantation site of bio-MEMS, blue: cell nucleus (4',6-diamidino-2-phenylindole), red: macrophage. Macrophage accumulation was observed at 1 week (Day 7: left) but it disappeared at 4 weeks (Day 28: right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The basic unit of the MEMS technique is the micrometer (μm); MEMS technology was initiated in the early 1960s to develop ultrapressure sensors [9,10]. In our study, we used microelectrodes prepared by the MEMS process to overcome the limitations of the equipment for monitoring changes in bladder volume in the urological experimental setting with small animal models.

With regard to the site of graft, some studies have been conducted of grafting the sensor inside the bladder [11]. However, such grafting may cause secondary problems, such as cystitis or stones [12]. Therefore, we monitored the changes in bladder volume by placing the MEMS electrodes on the external surface of the bladder.

Several variables may be relevant to the real-time monitoring of changes in bladder volume. Particularly, the shape of the bladder varies not only depending on the urine volume within the bladder but also depending on age, gender, the thickness of the bladder, and bladder activity. Therefore, one mathematical formula cannot represent the entire bladder volume precisely [13,14]. Thus, monitoring the bladder volume by use of a device can result in errors. On the other hand, when catheters are used, the volume within the bladder can be measured accurately. Nonetheless, from an experimental aspect, catheters have shortcomings in that they are inconvenient, may exert effects on other equipment, and frequently result in the introduction of artifacts. Furthermore, they can cause injuries to the bladder or urethra, which could subsequently affect the behavior of the urinary bladder during the experiments.

Simplification of the formula for volumetry could result in experimental efficiency and convenience, as was shown in a previous study in which the volume of the urinary bladder was estimated through measurement of ventrodorsal direction [2]. Similarly, in the present study, the shape of the small bladder filled with solution was similar to a sphere. By use of the distance converted from capacitance measured by the MEMS electrode, the bladder

volume was estimated by applying the formula for the volume of a sphere. Unfortunately, to the point of 0.6-mL bladder filling, the saline volume and the estimated bladder volume showed a slight difference in our study. This may have been because before filling the bladder to a certain level, the shape may not have been a sphere. However, when the volume of actually injected saline was more than 0.6 mL, the mean of the volume of injected saline and the measured bladder volume showed no statistical differences. Although there was no statistical difference between the mean infused volume and the mean estimated volume from the point when infused volume was 0.6 mL, the mean estimated bladder volume had a very slight tendency to increase from the point when infused volume was more than 1.8 mL. This might have been the effect of diuresis coming from the animal itself because we noted that the slower we infused the saline into the urinary bladder the higher the estimated bladder volume was. Therefore, we may achieve more precise results if there is no diuretic effect. Thus, the bladder volume measured through the MEMS electrodes could be considered to reflect the volume of injected saline. Therefore, a real-time bladder volume monitoring device using MEMS electrodes can accurately predict the excreted and residual urine volume in a small animal model.

The biocompatibility of a device is important for a device to be applicable clinically and for chronic implantation in a laboratory animal for long-term study. Several biomaterials have been investigated for implantation in humans, one of which is PDMS [15]. PDMS and its derivatives are well known as biosuitable materials. Generally, once a foreign body is inserted into a living subject, protein adsorption to the surface of foreign body and provisional matrix formation around it will occur within a few days, and then, subsequent inflammatory reaction will occur [16]. At this point, macrophage is the most important cell, so many scientists use ED1 and CD4 as macrophage markers to evaluate the biocompatibilities of their devices [17]. We evaluated the biocompatibility of PDMS to clarify its safety in experimental urology. The MEMS electrodes packed with PDMS showed reliable biocompatibility by means of ED1 staining.

Neurogenic bladder can develop as a result of aging, endocrine diseases such as diabetes, systemic vascular diseases, neurological diseases and injuries, and complications after pelvic surgeries [18,19]. Neurogenic bladder is a relatively common problem in the urological field. Regardless of its type, treatments are not easy, and cases developing secondary complications are not rare. For patients showing detrusor underactivity or acontractile neurogenic bladder, if the urination time is regulated properly, complications such as bladder calculus caused by chronic urine retention and the overexpansion of the bladder, infection, and gradual deterioration of renal function can be prevented. In such patients, urination is not normal, and thus the bladder is expanded above the normal bladder volume, or after urination, excessive residual urine remains. The treatment for detrusor underactivity or acontractile neurogenic bladder is for patients to regularly perform self-catheterization together with medical treatments to prevent the overexpansion of the bladder and to prevent the retention of residual urine. Nonetheless, in each individual, the produced urine volume may vary depending on food intake and metabolism. Thus, if methods allowing the real-time monitoring of bladder volume are available, the appropriate time to perform self-catheterization could be determined, which would aid in treatment. The *in vivo* bladder volume sensor analyzed in this study can measure real-time bladder volume in small animal models. However, further studies with chronic implantation of the device for collecting long-term data in the same animal model and with implantation of the device in large animal models are required to apply the implantable device clinically.

Limitations of this study

We did not evaluate our device at 1 week and 4 weeks whether the sensor still well working or not. Further longitudinal studies would be required to enhance the value of the device.

When urodynamic testing is performed in small animal models, the real-time monitoring of bladder volume together with the measurement of intravesical pressure can provide useful information for understanding bladder function. The *in vivo* bladder volume sensor introduced in this study can be used in a small animal model, and its installation is simple. Thus, if it is used together with conventional urodynamic study methods, it is thought to be a very useful device for monitoring bladder volume in an experimental setting. In addition, owing to its biocompatibility, the device could be chronically implanted in a conscious ambulating animal to perform a novel longitudinal study for a specific purpose.

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