Expandable and implantable bioelectronic complex for analyzing and regulating real-time activity of the urinary bladder

Tae-Min Jang 1*, Joong Hoon Lee 1*, Honglei Zhou 2,3†, Jaesun Joo 4, Bong Hee Lim 5, Huanyu Cheng 2, Soo Hyun Kim 1,6, Il-Suk Kang 7, Kyu-Sung Lee 4,5,8†, Eunkyoung Park 4,8†, Suk-Won Hwang 1†

Underactive bladder or detrusor underactivity (DUA), that is, not being able to micturate, has received less attention with little research and remains unknown or limited on pathological causes and treatments as opposed to overactive bladder, although the syndrome may pose a risk of urinary infections or life-threatening kidney damage. Here, we present an integrated expandable electronic and optoelectronic complex that behaves as a single body with the elastic, time-dynamic urinary bladder with substantial volume changes up to ~300%. The system configuration of the electronics validated by the theoretical model allows conformal, seamless integration onto the urinary bladder without a glue or suture, enabling precise monitoring with various electrical components for real-time status and efficient optogenetic manipulation for urination at the desired time. In vivo experiments using diabetic DUA models demonstrate the possibility for practical uses of high-fidelity electronics in clinical trials associated with the bladder and other elastic organs.

INTRODUCTION
Urination is an important physiological process that excretes wastes present in the body, managed by the urinary system—kidneys, ureters, bladder, and urethra—responsible for regulating blood pressure and pH and adjusting or maintaining levels of electrolytes and metabolites (1–3). The urinary bladder, a primary organ in charge of urination, serves as a container to store and release urine with cyclic, periodic expansion and contraction movements, which are mechanically controlled by the detrusor muscle in the wall of the elastic bladder (3, 4). In particular, detrusor underactivity (DUA) or underactive bladder (UAB) refers to a symptom of prolonged and/or incomplete bladder voiding due to a contraction of reduced strength, which is often observed in various neurogenic and myogenic disorders or failures, although understanding of the accurate origin and mechanism of the occurrence is not sufficient (5, 6). Current therapeutic interventions including intermittent self-catheterization and pharmacological and surgical approaches provided poor clinical efficacy for a variety of reasons such as urinary tract infection (UTI), off-target effects, and others (7–9). Only the intermittent catheterization of inserting a thin tube into the urethra is known to be an effective method to empty bladder but still has limitations of being easily exposed to UTI or experiencing periodic discomfort and pain, especially during insertion (5, 10, 11). Thus, the desirable direction would lie on the pursuit of a new type of system that enables us to collect continuous, reliable bladder information and to provide fundamental treatments incorporated with existing technologies.

Mechanically soft, stretchable architectures and intrinsically deformable materials, or biologically safe and degradable elements in some cases, offer versatile opportunities in many respects over conventional systems where physical rigidity and long-term operation are the first consideration (12–14). Past works, particularly in cases that integrated with the human body, demonstrated that minimization of mismatch in mechanical properties and exploitation of materials present in the body provide critically important benefits, along with examples ranging from implantable electronics to wearable electronics and soft robotics (14–16). Here, we describe an expandable electronic and optoelectronic system that is designed to monitor and stimulate the elastic urinary bladder with substantial volume fluctuations. Characteristics of electrical and optical components present sufficient functional performances for studying the movements of the bladder. Experimental and theoretical results describe the underlying behaviors of the whole system and its responses to mechanical motions. In vivo experiments provide validations of our system for practical use in biological organs.

RESULTS AND DISCUSSION
Overview of the expandable and implantable bioelectronic complex
The occurrence of prolonged voiding dysfunction originates from a number of reasons, including bladder outlet obstruction and myogenic and neurogenic impairments, potentially causing a series of different types of possible complications to associated functional organs or tissues (17–22). Figure 1A illustrates the normal process of urination (left) and critical phenomena and conditions including...
recurrent UTI or chronic kidney failure from vesicoureteral reflux and changes in detrusor structure, e.g., noticeable thickening of the bladder wall (DUA), that may happen to the urinary system because of the abnormal activity of the urinary bladder, i.e., UAB (right) \((5, 6, 22, 23)\). Here, we propose an expandable electronic and optoelectronic complex that is capable of monitoring bladder conditions in real time through various parameters (strain, temperature, and electrical activity) and stimulating genetically modified bladders to induce urination on demand. The overall system consists of a combination of two components: (i) A web-type scalable elastomeric framework, electronic web (E-web), enables us to not only envelop the urinary bladder across the entire surface but also maintain intimate contacts with the distensible sac while undergoing substantial volume changes. (ii) A narrow, thin, and stretchable electronic strip, electronic thread (E-thread), includes sensors, electronics, and micro–light-emitting diodes (µ-LEDs) for comprehensive functional activities (Fig. 1B). The systematic scenario involves that changes in volume or temperature when the bladder was filled or discharged with urine can be monitored with strain gauges, temperature meters, and electromyogram (EMG) electrodes, and

---

**Fig. 1. Soft, expandable electronic complex as an integrated system for continuous monitoring and optoelectronic stimulation for voiding dysfunction of the urinary bladder. (A)** Schematic diagram illustrating the periodic process of bladder emptying and filling in the normal urination system (left) and a causal factor of bladder dysfunction such as the UAB, and its possible risk or damage (right). Loss of bladder control associated with myogenic issues in the bladder causes incomplete voiding and/or urinary retention, eventually leading to critical damage to the kidneys when urine is forced back into them. **(B)** The overall operational process of recording and activating the repetitive motions of the bladder through implanted expandable electronics. The electronic complex consists of two key modules, i.e., an E-thread and electrically inactive web: Electronic and optoelectronic components formed on a thin, stretchable thread; a spiderweb-like polymeric framework was coupled to the bladder to maintain intimate contacts. **(C)** Photograph images of the soft electronic complex integrated into the urinary bladder in a rodent model, with a switched-on image of optical components in the inset. Photo credit: Tae-Min Jang and Joong Hoon Lee, Korea University.
feedback on the resulting values enabled optical stimulation to induce urination at desired times. The size of the bioelectronic complex was determined to fit the contraction state of the urinary bladder, which enabled good adherence to the bladder despite the cyclic occurrence of expansion and contraction motions. The rationale of the design strategy was guided partly by the ability to simply reconfigure or modify the E-web design and adjust or arrange electronic devices in the E-thread, according to different sizes and shapes of the bladder or human organs with massive volume changes and mechanical movements in future works. Images of the expandable system integrated onto an animal model appeared in Fig. 1C, with a magnified view using turned-on μ-LEDs in the inset. (Detailed information on fabrication processes and layer structures appears in figs. S1 to S3.)

**Optical and electrical characterization of the E-thread**

Maintaining seamless contacts through conformal lamination is critically important for achieving high-quality, accurate information when electronics integrated into the bladder with characteristics of the time-dynamic and curvilinear surfaces (15, 16). Figure 2A and fig. S4 show representative images of the E-thread with various electronic components and optoelectronic devices in stretchable configurations. Each component was evenly distributed across a total of seven islands, allowing the overall components to effectively operate over the entire surface of the urinary bladder. The small-size LEDs (dimension, 270 μm by 220 μm) enable us to provide cell type-specific stimulations with high spatial resolution of ~0.06 mm², and this resolution can be improved to reduce potentially undesirable secondary effects via light delivery (24). Figure 2B provides current-voltage (I–V) characteristics and output power density of a pair of μ-LEDs whose performances are sufficient to stimulate optogenetically engineered bladders (25, 26). Considering the overall thickness of the human bladder, which is approximately 0.780 ± 0.230 mm (27), the penetration depth (~1 mm) of blue light at 470 nm has the potential to be sufficiently used in clinical trials (28). A total of eight LEDs arranged into four groups on the electronic bank-like architecture enabled us to efficiently excite the whole muscular organ compared to approaches of stimulating specific direction or anterior wall of the bladder (29). Invariant properties of the emissive components during the repetitive activity of the urinary bladder are crucial for consistent activation of the LEDs during substantial volume changes (about three times) of the urinary bladder. Figure 2C provided a set of images of the E-thread at relaxed and stretched (60%) states, similar to the expanded radius rates of the urinary bladder (volume change fraction, 300%). Non-uniform brightness of the μ-LEDs, due to the difference in the resistance of electrical interconnects, can be improved by adjusting the thickness and the dimension of the conductors. After 3000 times of repeated stretching tests of the E-thread under tensile strain of 60%, the resulting optical power density exhibited no significant alterations on performance (Fig. 2D). The stimulation efficiency could be maximized in combination with reflectors of an ultrathin silver (Ag) film (300 nm) and white-dyed silicone elastomer to collect unintended scattered light (Fig. 2E). Here, the silver, which is prone to be easily oxidized, was encapsulated with a polyimide (PI) layer to protect it from the air or other effects. The resulting reflectance in Fig. 2F was found to increase approximately eight times near a wavelength of 470 nm [activation wavelength of channelrhodopsin2 (ChR2)], compared to that in the absence of reflectors. A similar analysis of output power density appeared in fig. S5. While the μ-LEDs on the E-thread are designed to deliver light to the bladder for optogenetic neuromodulation, their thermal effects also need to be minimized to avoid adverse thermal damage. Although the high thermal conductivity of the Cu and Ag in the E-thread helps ensure efficient dissipation of heat associated with the operation of μ-LEDs, the thermal output power from continuous illumination still leads to a temperature increase in the bladder tissue. In the finite-element models, four pairs of μ-LEDs are evenly distributed on the outer surface of the bladder. The maximum output power density of a pair of μ-LEDs is 2.4 mW/mm². As activation of μ-LEDs leads light with a power density of 0.9 mW/mm² to pass through the full-thickness bladder wall, the thermal output power becomes 1.5 mW/mm². While the temperature increase in the μ-LED saturates to 0.338°C after 480 s, the peak temperature increase of bladder tissue is below 0.2°C (Fig. 2G and fig. S6). Since the μ-LEDs are only activated for 5 s in the experiment, the peak temperature of the bladder tissue only increases to 0.0625°C at 5 s, indicating a negligibly small change in temperature in vivo.

Collections of physical and mechanical parameters associated with changes in the distensible bladder enable us to detect abnormal signs and/or anticipate appropriate times to urinate. Figure 2 (H to J) presents optical images and electrical properties of individual monitoring components. Doped single-crystalline silicon nanomembranes (Si NMs) can serve as strain gauges with a gauge factor (GF) of ~1.5, to estimate the amount of urine occupied in the muscular container (Fig. 2H). We note that the low GF originated from the filamentary serpentine bridges releasing the applied strain, and a typical GF from strain gauges in an isolated form was ~40, which is comparable to the value of Si NM–based strain sensors (fig. S7) (30). EMG electrodes can provide electrical activities of the bladder through the behaviors of relaxation and contraction in the detrusor muscle that primarily controls periodic bladder movements. In vivo measurements using the mouse bladder in Fig. 2I exhibited a distinct difference when urine was stored (filling) and released (voiding), suggesting the capability to manage mechanical deformations of the elastic bladder in conjunction with the strain gauges (3). Since the mechanical expansion of the bladder arises from the storage of urine, slight changes in the temperature of the bladder surface could provide useful information. Figure 2J presents an image of platinum (Pt)–based resistive temperature meter and linear response of electrical properties to temperatures, with a thermal coefficient of resistance of ~0.0016.

**Theoretical and experimental studies of the mechanical influences of the electronic complex**

Because of the bladder’s elastic nature, integration of an electronic system into the compliant, muscular organ requires a system design with mechanical features similar to the bladder within a level that does not interfere with the bladder’s natural motion. The feasibility of the expandable electronic complex was investigated using an artificial sac with shapes and properties similar to those of the actual bladder (fig. S8). Figure 3A presents a series of images of the expandable electronics integrated with the artificial model during the process of empty and full states (change in volume of ~1 ml), controlled through expansion and contraction with regulations in pressure (gas) and temperature (liquid). The images revealed that the choice of materials and the system configurations allowed the expandable system to maintain stable, conformal contacts with the...
artificial sacs while undergoing extreme transformations (~300% volume change). Figure 3 (B and C) exhibited stable, repeatable electrical responses of integrated strain gauges and temperature meters to cyclic alterations of volume (0.1 to 1.1 ml) and temperature (24° to 32°C) of the elastic sacs. The results suggested a possibility for monitoring the states of the actual bladder in biological experiments using animal models and, consequently, for triggering suitable onset timings of urination. Implanted images of the animal model and detailed calibration approaches appeared in figs. S9 and S10 (31). Comparisons of mechanical characteristics of the actual bladder tissue with different geometries (straight) including our approach (serpentine) are of great importance in terms of incorporation...
Fig. 3. Mechanical influences of the electronic complex on the urinary bladder. (A) Images of artificial distensible sacs wrapped with the expandable electronics, emulating the repetitive expansion (300% in volume) and contraction motions of the bladder. (B) and (C) Evaluations on the ability to monitor mechanical deformations (B) and temperature changes (C) during cyclic movements of filling and emptying through the artificial sac. (D) Stress-strain curves of the expandable network with different configurations (blue, straight; red, serpentine) and tissues (black) from the mouse urinary bladder. (E) and (F) Calculated elastic strain distributions of the E-webs, pressure distributions (E), and average pressure changes (F) at the outer surface of bladder tissue encircled by two E-webs (blue, straight; red, serpentine) from initial contracted state $V_0$ to two times ($V = 2V_0$) and three times ($V = 3V_0$) of its initial state. (G) Relationship between the elastic strain and volume change of the bladder during the first six expansion and contraction cycles. (H) to (J) Quantification of basal pressure (H), ICI (I), and bladder voiding efficiency (J) from mice implanted with various forms of the electronic complex (black, sham; red, serpentine; blue, straight). Photo credit: Tae-Min Jang and Joong Hoon Lee, Korea University.
into real organs (fig. S11) (15). Measured stress-strain curves of individual elements indicated that the E-web configuration was nearly identical to the actual bladder in the mechanical aspect (Fig. 3D). On the basis of these results, we estimated the mechanical effects of the electronic complex on the bladder model through a linear elastic model. In addition, the results with the use of the non-linear Mooney-Rivlin and Ogden hyperelastic models for the electronic complex and bladder tissue, as in the literature report (32), exhibit negligible changes, further justifying the choice of the simple linear elastic model in the following study unless specified otherwise. Applying the radial displacement to the bladder tissue in the static, general analysis simulates its uniform expansion. Quantitative analysis of the pressure associated with our electronic complexes on the bladder tissue during expansion reveals the dependence of the pressure on the material and design parameters of the electronic complex (Fig. 3E). As the bladder integrated with the serpentine (or the pressure on the material and design parameters of the electronic complex) extends on the bladder tissue during expansion reveals the dependence of the pressure associated with our electronic complex. As the bladder expanded with the coefficient of friction of 0.3 (fig. S15B). Therefore, the cumulative change of the sensors position in the serpentine form is only about 3% along the vertical direction after repeated cycles. Similar tests with the serpentine geometry with the coefficient of friction of 0.2 appeared in fig. S16. In addition, the strain distribution within this small region indicates negligible variations, which is confirmed by the nearly same elastic strain during the periodic movements of the bladder over the first six cycles (Fig. 3G).

Validations of in vitro experiments and theoretical models suggested the capacity to assess influences of the system designs on bladder functions using animal models. Figure 3 (H to J) shows the results of comparative analyses of several parameters associated with the bladder conditions after being implanted into mice with different configurations of the electronic complex. Increased basal pressures in the mouse bladder were observed in test samples with the straight geometry compared to those of a sham-operated control group, while no difference was observed in the serpentine geometry (see fig. S17 for detailed temporal traces). This behavior suggests that mismatches in the mechanical moduli between the elastic tissues and implanted devices created extra pressures onto the bladder, i.e., increases in the basal pressure (Fig. 3H). The elevated pressure contributed to decreases in the intercontraction interval (ICI) (Fig. 3I), leading to reduced voiding efficiency of the urinary bladder (Fig. 3J). According to the simulation results, the values of the contact pressure during various states of the expansion are much smaller than the sensation threshold of the tissue, which will not induce tissue lesions (35, 36). Additional inspections provided a range of appropriate basal pressures (1.604 to 3.418 mmHg) that enable the electronic complex to stably integrate with the bladder, without disturbing the normal physiological activity of the bladder (fig. S18).

**Evaluation of the electronic complex in the mouse disease model**

The prevalence of DUA is shown high among patients with diabetes mellitus (37, 38). In particular, the continued hyperglycemia, which is associated with oxidative stress and polyuria, can result in atony or DUA in the later stage of diabetes (38, 39). This motivated us to exploit an animal model of diabetes to assess the functionalities of the expandable electronic and optoelectronic complex. The diabetic DUA models were developed by a single injection of streptozotocin (STZ; 150 mg/kg), resulting in decreased body weight and increased blood glucose levels compared to a control group (fig. S19) (40, 41). Histological evaluations of bladder tissues exhibited significant hyperplasia of the detrusor smooth muscle and urothelium in the bladder wall, one of the representative features of DUA (Fig. 4A and fig. S20) (41). Comparison studies of the DUA model with a control group using cystometry reflected the well-formed disease models with the impaired contractile ability through ICI, post-void residual volume, and bladder voiding efficiency (fig. S21). To modulate the function of the targeted organ with a light-induced optogenetic system, adeno-associated virus (AAV) encoding ChR2 tagged with an enhanced green fluorescent protein (eGFP) [AAV9-CAG-hChR2(H134R)-eGFP] was injected into a region between layers of the detrusor muscle and adventitia in the bladder (fig. S22). Images of confocal microscopy on cross sections verified robust ChR2 expression in areas of the detrusor muscle with uniform distribution (Fig. 4B). Here, the AAV has been used in clinical trials for a
number of diseases because of their nonpathogenic nature, while no successful stories of bladder-related transfection with the viruses have been reported previously (42–44). On the other hand, herpes simplex virus and adenovirus have been popular viral vectors in previous researches including the bladder. However, potential availability to patients or the general public is restricted because of the threat causing other diseases (29, 32). Additional functional examinations were executed through ex vivo tests on isolated bladder strips from the ChR2-expressing urinary bladder (fig. S23). KCl (62 mM)–induced maximum contractile response via cytometry was lower than that of a control group, and optical responses under illumination of blue light (470 nm, 0.9 mW/mm²) for 5 s was restored to the level of ~80% of the maximum contractile force (fig. S24). We note that this reduced efficiency might be attributed to incomplete ChR2 expression over the entire detrusor muscle (45). Figure 4C describes overall procedures of implantation and operation of the expandable electronics with additional tools using the virally mediated disease model (left) and an implanted system at the initial phase (middle) with a demonstration of the fully implanted system with light-induced voiding procedures (right).

To demonstrate bidirectional manipulations of the bladder activities with the expandable electronic and optoelectronic system, various monitoring and stimulating parameters were simultaneously recorded through in vivo experiments. The expandable electronics communicated with an external control system via a wired connection for continuous, real-time recordings and analyses (fig. S25A).
The system was sterilized with ethylene oxide gas and then integrated with the urinary bladder of mice in accordance with the guidelines of the Institutional Animal Care and Use Committee protocols of the Samsung Medical Center. Figure 4D shows a dataset of dynamic variations over time of intravesical pressure (green), strain (black), EMG (blue), temperature (purple), and micturition weight (magenta) as a result of the process of urination in the bladder. Periodic, repeated optical stimulations with the integrated μ-LEDs (red) induced notable physical and electrical changes in the intravesical pressure, strain, and EMG in response to the contractile force of the bladder for voiding of urine, followed by gradual increases in the pressure and strain as the bladder began to relax and store urine. Particularly in the DUA model, the EMG signals, generated by contraction of the detrusor muscle, showed inevitable fluctuations even before urinations, indicating that voluntary initiations were unable to complete because of reduced contractile capability, where similar behaviors were observed in the intravesical pressure. The recorded temperature exhibited a slightly different behavior, which was maintained with a sudden rise as the urine was stored above a certain amount because of the position of the temperature sensor installed in the E-thread, followed by a steep fall with urination. A customized protocol automatically recorded biological parameters and manipulated optical stimulation in a real-time mode based on a flowchart as a sequence of operation. (fig. S25, B and C). The cumulative increase in micturition weight from the analytical balance demonstrated that urinations were normally induced by optical stimulations. Figure 4E presents an enlarged view of the measured signals in a specific temporal range. Stimulation parameters including optical power and illumination duration were determined in the optimized condition (0.9 mW/mm², 5 s) to effectively activate ChR2-expressing detrusor muscle and minimize unexpected effects that might alter neural activities even in non-expressed cells (fig. S26). With the given values, we were able to derive appropriate contraction pressure within the physiological range of bladder voiding pressure of mice (18 to 25 mmHg) (46). We note that pulsed optical stimulation can be useful to improve stimulation efficiency through precise control of frequency (25, 29). Examinations with cystometric parameters, histological assessments, and immune profiles of blood cells on biocompatibility of the integrated expandable electronics at 2 weeks after implantation appear in figs. S27 to S29.

The concepts, materials, and system design strategies reported here suggest an approach to enable continuous electrical recording and optoelectronic manipulation of the physical/mechanical behaviors of the elastic bladder. The expandable electronic complex incorporated with various components in optimized configurations demonstrated reliable, durable performance through maintaining excellent coupling with the biological organ, which were validated by the theoretical models. In vivo operations using the animal models of DUA confirmed the capability of the electronic complex for possible applications to clinical uses and to other elastic organs.

**MATERIALS AND METHODS**

**Fabrication of a soft, expandable electronic complex**

Spiderweb-like polymeric structures were prepared using a photo-curable epoxy (SU-8 100, MicroChem Inc., USA) as a master mold and polydimethylsiloxane (SYLGARD 184, Dow Corning, USA) as a replica mold. The original patterns were then transferred to a target substance, e.g., a silicone elastomer (Ecoflex 00-30, Smooth-On, USA), and curing and demolding procedures completed reproducible, desired architectures. For E-thread–like filaments, interconnects (Ti/Cu, 10/500 nm) for μ-LEDs were formed by electron-beam evaporator onto layers of a poly(methyl methacrylate) (MicroChem Inc., USA) and diluted PI (D-PI; Sigma-Aldrich, USA) coated silicon carrier wafers. Another D-PI layer was coated for interlayer dielectrics, and dry etching opened contact regions for μ-LEDs. After an undercutting process in acetone, the entire layers were transferred onto a layer of white-dyed Ecoflex 00-30 (~70 μm) and integrated with μ-LEDs (TR2227, Cree Inc., USA) using a silver epoxy with curing at 120°C for 20 min. Flexible anisotropic conductive film (ACF) (Elform) constructed electrical connections to external hardware, and an additional layer of Ecoflex 00-30 (~70 μm) was coated for interlayer dielectrics. Next, monitoring components such as a strain gauge (Si NM, 300 nm), temperature sensor (Ti/Pt, 5/50 nm), and EMG sensor (Ti/Au, 10/300 nm) were separately fabricated. Doping, photolithography, deposition, and etching procedures allowed to monolithically fabricate individual electronic elements, and the whole device was transfer printed onto the previously formed Ecoflex. Electrical connections via flexible ACF cables completed the fabrication process of the E-thread.

**Mechanical characteristics of the urinary bladder and soft electronic systems**

Stress-strain curves of bladder tissues (extracted from a 10-week-old animal model) and three different configurations (film, straight, and serpentine) of an expandable electronic system were prepared with dimensions of 10 mm by 5 mm by 0.1 mm. Each sample was examined at a speed of 0.1 mm/min with a 20-N extension force using a universal electromechanical testing machine (Instron 5900 series).

**In vitro experiments of an expandable electronic system using an artificial bladder model**

A synthetic bladder model was constructed using a three-dimensional printed scaffold with a silicone elastomer (Ecoflex 00-30) to evaluate the electronic system’s ability to monitor diverse physical/mechanical parameters that may occur in the urinary bladder. Two catheters were placed on each side of the artificial bladder to serve as an inlet and outlet, and a solenoid valve was combined to the outlet to control the bladder system. Injections/discharges of air and hot/cold water emulated periodic contractions/expansions in the real bladder, enabling us to monitor continuous, real-time changes in electrical/mechanical parameters (strain and temperature) using the integrated electronic system.

**Diabetic bladder dysfunction models**

Nine-week-old male C57BL/6 mice (24 to 27 g) were housed at constant room temperature with 12-hour light/12-hour dark cycles. Food and water were available ad libitum. The animals were divided into two groups, namely, control and diabetic. Diabetic mice received a single intraperitoneal injection of STZ at 150 mg/kg dissolved in citrate buffer (0.1 M, pH 4.6). Control mice were treated identically except that a similar volume of buffer was injected instead of STZ. Blood samples were taken from the tail 48 hours after administration of STZ to confirm the induction of diabetes mellitus. Mice with blood glucose levels above 300 mg/dl (as measured with the Accu-Chek Advantage blood glucose monitoring system; Roche Diagnostics, USA) were considered to be diabetic. Animal weights and blood glucose measurements were obtained once a week. All
experimental comparisons of diabetic and nondiabetic mice were made 22 to 25 weeks following STZ administration.

**Adeno-associated viral constructs and animal transfection**

To achieve expression of exogenous hChR2 (H134R) opsin in the mouse bladder, we injected AAV serotype 9 (AAV9) vector packaged with opsin directly into the detrusor muscles of diabetic and control mouse bladders. In vivo-grade AAV9-CAG-hChR2(H134R)-eGFP viral constructs were titrated at $3.3 \times 10^{13}$ GC (genome copies)/ml (Vector Biolabs, USA). Mice, 16 to 18 weeks after administration of STZ, were anesthetized by inhalation of 2% isoflurane (v/v), and body temperature was maintained at 37°C using a heating pad. Viral injections were performed according to the published method (29). The volumes for control and diabetic mice were injected with 33 and 55 μl, respectively. The injections were divided into 6 to 10 times of 5 μl each to completely cover the entire bladder surface. At 6 weeks after surgery, each animal was used for subsequent experiments.

**In vivo animal experiments**

In vivo experiments were performed in mice under decerebrated and unanesthetized conditions according to the published method (47). The animals were anesthetized with isoflurane (2 to 3%) in oxygen (flow rate, 0.3 liters/min) during surgery before the decerebration. Both carotid arteries were ligated before intubation. The trachea was cannulated with a polyethylene tube (PE-90, Clay-Adams, Parsippany, NJ) to facilitate respiration. A catheter (PE-50) was inserted into the bladder through the dome, and a suture was tightened around the collar of the catheter. Then, the expandable electronic complex was integrated with the bladder. The head skin was incised in the midline with a scalpel and the skull and forebrain were removed with a fine rongeur and a blunt spatula, respectively. Isoflurane was then discontinued. After no further intracranial hemorrhage was visually detected, both lateral flaps of the incised head skin were sutured together. More than 2 hours after the surgery, evaluations on physiological parameters from the E-web, cystometrograms, and weighing of excreted urine were simultaneously performed with saline instillation at a rate of 1.2 ml/hour. For optical stimulations, the exposed bladder was subjected to a 5-s pulse of blue light (450 nm, 6 mW/mm²) stimulation. The output signals from cystometry and weights of voided urine were acquired and processed at a sampling rate of 200 Hz using a data acquisition (DAQ) system (PowerLab 8/30, AD Instruments). Recorded values via the electronic complex were also achieved using a Keithley 2636B SourceMeter and a customized program (LabVIEW, National Instruments).

**DAQ procedure**

E-thread was linked to an external manipulation system through a wired connection. On the basis of a customized software program (LabVIEW 2015, National Instruments, USA), we recorded vital signals from monitoring components (temperature meters, strain gauges, and EMG electrodes) in a continuous, real-time mode and regulated optical stimulations to induce urination. In detail, continual changes in resistance/potential of temperature meters, strain gauges, and EMG collectors were recorded by source meter (Keithley 2636B, USA), and measured EMG signals were amplified by differential ac amplifier (Model 1700, A-M Systems, USA), and then, the individual dataset was collected by a DAQ system (USB-6255, National Instruments, USA) with a sampling frequency of 1 kHz. The resulting information was used to regulate/trigger the optogenetic stimulations through a customized algorithm, and a preset potential was applied to arrays of μ-LEDs on the E-thread via analog output channels in the DAQ system.

**Customized algorithm for regulation of optogenetic stimulation**

A customized algorithm based on the commercial software (LabVIEW 2015, National Instruments, USA) continuously recorded biological parameters and manipulated optical stimulation in a real-time mode according to logical pathway of a flowchart as a sequence of operation (fig. S25). For example, sudden rise (threshold value, 36.5) of temperature according to increasing volumes of urine was confirmed at early stage, and certain changes in resistance of strain gauge was measured (threshold value, 0.4) on the basis of the correlation results from in vitro tests. The system, then, checked frequent fluctuations in EMG signals (calculated threshold root mean square value, 0.3) of the detrusor muscle, which appears when mice exerted pressure on the bladder for urination (15 s). The entire procedure was designed in a way that recorded biological parameters as output signals were continuously compared to satisfy the specified threshold values in each stage before proceeding to the next stage. After urination was induced by optical stimulation, the protocol was returned to the first stage of the flowchart.

**Morphological evaluation and immunohistochemistry**

Mice were perfusion fixed with a fixative solution [4% paraformaldehyde (PFA)] through the circulatory system. Isolated whole bladders were fixed in the 4% PFA solution overnight, and paraffin was embedded 22 to 25 weeks following STZ administration. Experimental comparisons of diabetic and nondiabetic mice were confirmed at early stage, and certain changes in resistance of strain gauge was measured (threshold value, 0.4) on the basis of the correlation results from in vitro tests. The system, then, checked frequent fluctuations in EMG signals (calculated threshold root mean square value, 0.3) of the detrusor muscle, which appears when mice exerted pressure on the bladder for urination (15 s). The entire procedure was designed in a way that recorded biological parameters as output signals were continuously compared to satisfy the specified threshold values in each stage before proceeding to the next stage. After urination was induced by optical stimulation, the protocol was returned to the first stage of the flowchart.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/46/eabc9675/DC1

**REFERENCES AND NOTES**

1. C. J. Fowler, D. Griffiths, W. C. de Groat, The neural control of micturition. *Nat. Rev. Neurosci.* 9, 453–466 (2008).
2. A. Elbadawi, Functional anatomy of the organs of micturition. *Urol. Clin. North Am.* 23, 177–210 (1996).
3. W. C. de Groat, Integrative control of the lower urinary tract: Preclinical perspective. *Br. J. Pharmacol.* 147, 525–540 (2006).
4. N. Yoshimura, W. C. de Groat, Neural control of the lower urinary tract. *Int. J. Urol.* 4, 111–125 (1997).
5. M. J. Drake, J. Williams, D. A. Bijos, Voiding dysfunction due to detrusor underactivity: An overview. *Nat. Rev. Urol.* 11, 454–464 (2014).
6. N. Hoag, J. Gani, Underactive bladder: Clinical features, urodynamics parameters, and treatment. *Int. Neurol. J.* 19, 185–189 (2015).
7. T. Harada, K. Fushimi, A. Kato, S. Nishijima, K. Sugaya, S. Yamada, Demonstration of muscarinic and nicotinic receptor binding activities of distigmine to treat detrusor underactivity. *Biol. Pharm. Bull.* 33, 653–658 (2010).
P. Tenke, B. Köves, T. E. B. Johansen, An update on prevention and treatment on contractile detrusor management: Introduction of a new test. Sci. Transl. Med., 5, e1602076 (2017).

A. A. B. Lycklama a Nijholt, S. Siegel, U. Jonas, C. J. Fowler, M. Fall, J. B. Gajewski, M. M. Hassouna, F. Cappellano, M. M. Elhilali, D. F. Milam, A. K. Das, H. E. Dijkema, B.H.L., K.-S.L., and E.P. took responsibility for animal care, conducted immunohistology, and designed the research. T.-M.J., J.H.L., and I.-S.K. fabricated the devices and electronics. J.J., T.-M.J., J.H.L., and S.-W.H.

Author contributions: Data Sciences’ Roar supercomputer. H.C. also acknowledges the support from a Doctoral Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy (MI, Korea) and a National Research Fund grant funded by the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, Republic of Korea, the Ministry of Food and Drug Safety) (202012D30). Computations for this research were performed on the Pennsylvania State University’s Institute for Computational and Data Sciences’ Roar supercomputer. H.C. also acknowledges the support from a Doctoral New Investigator grant from the American Chemical Society Petroleum Research Fund (59012-D15) and NSF (ECRS-1933072). Author contributions: T.-M.J., J.H.L., and S.-W.H. designed the research. T.-M.J., J.H.L., and I.-S.K. fabricated the devices and electronics. J.J., B.H.L., K.-S.L., and E.P. took responsibility for animal care, conducted immunohistology, and

Acknowledgments: Funding: This work was supported by the Korea University, KU-KIST Graduate School of Converging Science and Technology Program, Technology Innovation Program (20002974) funded by the Ministry of Trade, Industry and Energy (MI, Korea) and a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (grant NRF-2017R1A1B101075027). E.P. acknowledges the support of the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, Republic of Korea, the Ministry of Food and Drug Safety (202012D30). Computations for this research were performed on the Pennsylvania State University’s Institute for Computational and Data Sciences’ Roar supercomputer. H.C. also acknowledges the support from a Doctoral New Investigator grant from the American Chemical Society Petroleum Research Fund (59012-D15) and NSF (ECRS-1933072). Author contributions: T.-M.J., J.H.L., and S.-W.H. designed the research. T.-M.J., J.H.L., and I.-S.K. fabricated the devices and electronics. J.J., B.H.L., K.-S.L., and E.P. took responsibility for animal care, conducted immunohistology, and

Jang et al., Sci. Adv. 2020; 6 : eabc9675 11 November 2020

10 of 11
cystometry experiments on mice. T.-M.J., J.H.L., J.J., B.H.L., E.P., and S.-W.H. performed in vivo measurements and analysis. S.H.K. and K.-S.L. provided clinical advice relevant to the study. H.Z. and H.C. performed mechanical simulations. T.-M.J., J.H.L., H.Z., E.P., H.C., and S.-W.H. wrote the manuscript. All authors reviewed and commented on the manuscript. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusion in this paper are present in the paper and/or Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 24 May 2020
Accepted 23 September 2020
Published 11 November 2020
10.1126/sciadv.abc9675

Citation: T.-M. Jang, J. H. Lee, H. Zhou, J. Joo, B. H. Lim, H. Cheng, S. H. Kim, I.-S. Kang, K.-S. Lee, E. Park, S.-W. Hwang, Expandable and implantable bioelectronic complex for analyzing and regulating real-time activity of the urinary bladder. Sci. Adv. 6, eabc9675 (2020).