Low-intensity extracorporeal shockwave therapy ameliorates diabetic underactive bladder in streptozotocin-induced diabetic rats


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Objectives
To evaluate the therapeutic effect of once-weekly low-intensity extracorporeal shockwave therapy (Li-ESWT) on underactive bladder (UAB) in the streptozotocin (STZ)-induced diabetic rat model.

Materials and Methods
In all, 36 female Sprague–Dawley rats were divided into three groups: normal control (NC), diabetes mellitus control (DMC), and DM with Li-ESWT (DM Li-ESWT). The two DM groups received an intraperitoneal 60 mg/kg STZ injection to induce DM. The Li-ESWT was applied toward the pelvis of the rats starting 4 weeks after STZ administration and lasting for 4 weeks. The Li-ESWT was given once weekly, with an energy flux density of 0.02 mJ/mm² at 3 Hz for 400 pulses. All rats underwent conscious cystometry, leak-point pressure (LPP) assessment, ex vivo organ-bath study, histology, immunofluorescence, and Western Blot analysis.

Results
Conscious cystometry revealed voiding dysfunction in the DMC group, whereas the DM Li-ESWT group showed significantly improved voiding function, reflected in a reduced post-void residual urine volume and increased LPP compared to the DMC group. Ex vivo organ-bath studies showed that Li-ESWT enhanced muscle contractile activity of the bladder and urethra during electrical-field stimulation and drug stimulation. Histologically, Li-ESWT significantly restored bladder morphology, reflected by a reduction in the intravesical lumen area and increased muscle proportion of the bladder wall. Western Blot analysis showed higher smooth muscle actin expression in the bladder wall in the DM Li-ESWT group compared to the DMC group. Immunofluorescence showed decreased nerve-ending distribution, and destroyed and shortened nerve fibres in the DMC group, and recovery of neuronal integrity and innervation in the DM Li-ESWT group.

Conclusions
In conclusion, Li-ESWT ameliorated UAB and urinary incontinence in the diabetic UAB rat model. The improvement appears to be the result of restoration of bladder and urethral structure and function by Li-ESWT. Li-ESWT is non-invasive and may become a better alternative therapy for UAB. Further investigations are warranted.

Keywords
low-intensity extracorporeal shockwave therapy, underactive bladder, diabetic bladder dysfunction, nerve innervation, muscle regeneration

Introduction
Diabetes mellitus (DM) is a highly prevalent metabolic disease and has various sequelae in multiple organs and systems. Diabetic bladder dysfunction (DBD) is one of the major sequelae, and its symptoms vary with the severity and progression of DM [1]. Patients with DBD express overactive bladder symptoms as increased daytime frequency and urgency in the early stage of DM, which subsequently transform to underactive bladder (UAB) in the late stage. UAB is the consequence of late-stage DBD with the symptoms of decreased bladder contractility and sensation, atonic bladder, increased post-void residual urine volume (PVR), and recurrent UTI [2]. Furthermore, half of diabetic
patients experience urinary incontinence (UI) after 10 years of DM progression [3].

DM treatment focuses foremost on controlling the blood glucose level, and then on managing DM-related complications of affected target organs and systems. Muscarinic stimulants, such as bethanechol, have been used to improve bladder contraction in diabetic UAB; however, these drugs can cause serious side-effects and sometimes produce unsatisfactory outcomes. Intermittent catheterisation is usually recommended for UAB to drain away residual urine, reduce UTIs, and prevent further kidney injury, but such treatments reduce the quality of life [4]. The pathomechanism of DBD with UAB has been investigated broadly. Polyuria and hyperglycaemia play different roles in the pathogenesis. Polyuria plays an important role in the bladder wall thickness in DBD [5]. Moreover, hyperglycaemia-induced oxidative stress products accumulate, cause damage to muscles, neurones, urothelium, and urethra [6], and result in the progression to UAB.

Several experimental treatment modalities have been proposed for enhancing bladder function. Sasaki et al. [7] used viral vectors to deliver the nerve growth factor (NGF) gene to the bladder wall, resulting in increased NGF levels and improved bladder function. Gopinath et al. [8] demonstrated the effect of smooth muscle transplantation into the diabetic bladder wall, which resulted in increased bladder contractile function and decreased PVR. A previous study by our laboratory demonstrated a satisfactory outcome with adipose tissue-derived stem cell therapy in DBD with UAB, through inhibition of apoptosis and promotion of vascular integrity [9]. However, practical clinical modalities with fewer side-effects are not available at present.

Low-intensity extracorporeal shockwave therapy (Li-ESWT) has been used as a clinical treatment modality in many types of diseases, including myocardial infarction and heart failure [10], bone fracture and tendinopathy [11], chronic soft tissue wounds [12], and skin ischaemia and skin grafts [13]. Li-ESWT has also been applied to a variety of urological disorders. For example, a systemic review of the effects of Li-ESWT on erectile dysfunction revealed encouraging results: amongst 14 studies including 833 patients from 2005 to 2015, Li-ESWT was found to improve erectile function, as measured by the International Index of Erectile Function (IIEF) and Erectile Hardness Score (EHS) [14]. Other urological diseases, such as prostatitis [15] and chronic pelvic pain syndrome [16], have also been shown to benefit from Li-ESWT. Regarding the application of Li-ESWT to DBD, a previous report from our laboratory showed a positive effect in improving overactive bladder by defocused Li-ESWT administered three-times per week [17]. To further evaluate (i) the therapeutic effects of Li-ESWT on UAB, which develops in the later stage of DM, and (ii) to explore whether reduced frequency of therapy can produce similar beneficial effects, we used a defocused Li-ESWT device applied once weekly in a streptozotocin (STZ)-induced diabetic rat model.

Materials and Methods
Animals and Experimental Design

In total, 36 female, 8-week-old Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA). The experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee of the University of California San Francisco. The rats were divided into three groups: normal control (NC), diabetic mellitus control (DMC), and DM with Li-ESWT (DM Li-ESWT). The DMC and DM Li-ESWT rats received a single intraperitoneal injection of 60 mg/kg STZ for DM induction. All rats were housed in a standard room with constant temperature and humidity, and a 12-h light–dark cycle. The rats had access to tap water and standard rat chow ad libitum. Blood glucose and body weight were measured at 6 h before STZ injection, 3 days after STZ injection, and then every week for the remainder of the study.

Li-ESWT

Li-ESWT was applied 4 weeks after DM induction and lasted for 4 weeks, with a once-weekly regime in the DM Li-ESWT group using the following parameters: 0.02 mJ/mm² energy flux density at 3 Hz for 400 pulses. Under isoflurane anaesthesia and with the rats in a prone position, the Li-ESWT probe, which contains a compact electromagnetic unit with a defocused shockwave source (LiteMed Inc., Taipei, Taiwan), was applied on the lower abdomen of the rats aiming toward the bladder and urethra. The sham procedures including the same anaesthesia exposure and recovery were performed for the NC and DMC groups for 4 weeks. After a 1-week washout period, the rats underwent conscious cystometry and leak-point pressure (LPP) assessment, followed by sacrifice and tissue harvest for the ex vivo organ-bath studies and molecular analyses.

Conscious Cystometry

At 24 h before conscious cystometry, the surgery for tube implantation was performed. Two polyethylene-90 (PE-90) tubes (Clay-Adam®) were placed in the intravesical and intraperitoneal regions, respectively, for pressure measuring. The next day, the rats were placed in the tunnel of the cystometry cage (Braintree Scientific®, Braintree, MA, USA). The two implanted tubes were connected to pressure transducers (Utah Medical Products®, Midvale, UT, USA) attached to a computer, and Labview 6.0 software (National Instruments®, Austin, TX, USA) was used to record the
pressure continuously. Simultaneously, an electric scale was also connected to the computer to record the voided volume. Normal saline was infused into the bladder at a rate of 0.1 mL/min using an infusion pump. After 20 min to allow stabilisation, a 1-h conscious cystometry was recorded.

LPP

Under urethane anaesthesia, the rats were placed in a supine position and at the level of zero pressure. After incision of the lower abdomen and exposure of the bladder, the bladder was filled halfway with normal saline through the bladder catheter. The bladder capacity was obtained by adding the PVR (obtained by intravesical catheter) to the voided volume. One half of the bladder volume was used to fill the bladder for the study. Using two cotton swabs, gradual pressure was applied to the bilateral sides of the bladder and then stopped immediately when urine leakage occurred from the urethral orifice. The pressure at which this leakage occurred was regarded as the LPP. The procedure was repeated five times to calculate the average LPP for each rat.

Ex Vivo Organ-Bath Study

The proximal two-thirds of urethra and bladder strips (one-third bladder length) were prepared in Krebs solution. The proximal two-thirds of urethra and bladder strips (one-third bladder length) were prepared in Krebs solution. The chambers were loaded with Krebs solution maintained at 37°C and supplied continuously with 95% O2 and 5% CO2. The tissue samples were linked to force-displacement transducers. A pair of platinum electrodes was placed on the bilateral sides of the tissue, and electronic stimulators were connected to the electrodes. For urethra, electrical-field stimulation (EFS) square-wave pulses were applied at 10–70 mA intensity and 0.2 ms duration with a 3-min interlude in the urethral pulse study. For urethral fatiguing stimulation, repeated multi-pulse EFS were applied at maximal intensity 70 mA at 5 Hz square-wave pulses for 5 min. For the bladder EFS study, a train duration of 10 s and an inter-train interval of 90 s were used, and frequency response curves were recorded at 2, 5, 10, and 20 Hz. Tetrodotoxin, a neurotoxin that blocks nerve stimulation, was added into the chambers before each EFS to ensure the contractions were directly from muscle and not nerve mediated. The drug stimulation studies were performed with 40 mM caffeine in urethra and carbachol in bladder. Muscle contractile activity (MCA) was calculated by using the equation: MCA (N/cm²) = [force (g) × muscle length (cm)] / [muscle weight (g) × 0.00981]. A Lab TRAX-4 data acquisition system (Myobath Tissue Bath system II®) was used for measuring the isometric tension.

Histology, Immunofluorescence, and Western Blot Analysis

Tissues were harvested immediately after the LPP study. The rats in each group were divided into two groups for either the histological or organ-bath study, which have different storage protocols. For the histological analysis, the tissues were fixed, embedded and sliced as routine. Haematoxylin and eosin staining and Masson’s trichrome staining were performed for histological evaluation. For immunofluorescence, primary antibodies were incubated with anti-s100 antibody and anti-SMA antibody (Abcam®, Cambridge, MA, USA). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen®, Carlsbad, CA, USA). For Western Blot analysis, the procedures for Western Blot analysis followed our established protocols [19]. The primary antibodies were incubated with β-actin antibody and anti-SMA antibody. Image analysis was performed with a ChemiImager 4000® (Alpha Innotech Corporation, San Leandro, CA, USA) to calculate the density of each protein band.

Image and Statistical Analyses

For histological-image analysis, five separate fields from each slide were recorded with a Retiga Q image digital camera and ACT-1 software (Nikon Instruments Inc.®, Nikon Corporation, Tokyo, Japan). Image quantification was estimated by Image-Pro Plus (Media Cybernetics®, Rockville, MD, USA). Numerical data were analysed with Prism 5 (Graph Pad Software®, GraphPad Software Inc., La Jolla, CA, USA) and expressed as mean ± standard error of the mean (SEM). To test for significant differences, multiple groups were analysed by t-test and one-way ANOVA. The Tukey–Kramer test was applied for post hoc comparisons. A P < 0.05 was considered to be significant for the comparison of the two groups.

Results

Biological Characteristics of the Rats

Weekly body weight measurements revealed significantly higher body weights for NC compared with DMC and DM Li-ESWT rats, at a mean (SEM) of 293.2 (4.8) g for the NC group vs 227.2 (6.8) g for the DMC group vs 231.1 (4.0) g for the DM Li-ESWT group (P < 0.05, at the 10th week) (Fig. 1). The difference in body weight between the groups became apparent 2 weeks after DM induction. DMC and DM Li-ESWT rats had significantly higher blood glucose levels compared with NC rats, at a mean (SEM) of 115.3 (3.0) mg/dL in the NC group vs 492.9 (15.0) mg/dL in the DMC group vs 499.0 (18.7) mg/dL in the DM Li-ESWT group (P < 0.05, at the 10th week). There were no differences in body weight and blood glucose between DMC and DM Li-ESWT rats.
Li-ESWT Improves Function of UAB in Diabetic Rats

Conscious cystometry revealed significantly different urinary patterns between NC and DM rats. Representative results for a 30-min recording interval for each group are shown in Fig. 2. The cystometric graph shows the regular and stable urinary patterns in NC rats; weak, irregular and unstable urination in DMC rats; and improved bladder function in DM Li-ESWT group. The DM Li-ESWT group showed improved voiding function, reflected by a shorter micturition interval, higher urinary frequency, lower voided volume, and higher maximal detrusor pressure without abdominal-strained urination. Parameters in cystometry showed the deterioration of bladder function in DMC compared with NC rats (Table 1). After Li-ESWT, DM Li-ESWT rats had significantly improved voiding function, reflected by a decreased PVR (mean [SEM] in the DMC group of 0.91 [0.23] mL vs 0.30 [0.07] mL in the DM Li-ESWT group; $P < 0.05$) and increased LPP (mean [SEM] in the DM Li-ESWT group of 31.3 [2.0] cmH2O vs 38.2 [1.8] cmH2O in the DMC group; $P < 0.05$) compared with DMC rats. Furthermore, there were other parameters in DM Li-ESWT group that showed improving trends but did not reach statistical significance, including decreased void volume, decreased micturition interval, and increased maximal detrusor pressure. Taken together, these parameters show deterioration of the voiding function to UAB as a consequence of DM, and its amelioration by Li-ESWT.

Li-ESWT Enhances Detrusor Contractility

In the ex vivo organ-bath studies for the urethra (Fig. 3), the DMC group showed significantly impaired MCA by EFS compared with the NC and DM Li-ESWT groups. The DMC group had a significantly lower percentage of MCA in the fatigue stimulation test compared with the NC and DM Li-ESWT groups, at a mean (SEM) of 62.3 (8.1)% in the NC group vs 38.1 (5.4)% in the DMC group vs 56.5 (1.9)% in the DM Li-ESWT group ($P < 0.05$). The DMC group had significantly impaired urethral MCA stimulated by caffeine compared with NC and DM Li-ESWT groups, at a mean (SEM) of 4732 (487) N/cm² in the NC group vs 1277 (83) N/cm² in the DMC group vs 3222 (694) N/cm² in the DM Li-ESWT group ($P < 0.05$). In the bladder study (Fig. 4), the DMC group showed significantly impaired MCA stimulated by EFS compared with NC and DM Li-ESWT groups. These organ-bath studies demonstrate that Li-ESWT restores the muscle contractile functions of the bladder and urethra impaired by DM.

Li-ESWT Activates Bladder Muscle Regeneration

Bladder wall remodelling was found in the DM and DM Li-ESWT groups (Fig. 5). The DMC rats exhibited the pathological changes of UAB in late-stage DBD, including chronic inflammation, tissue oedema, and muscle atrophy of the bladder wall, which are characteristics of diabetic UAB. After Li-ESWT, the DM Li-ESWT group exhibited a decrease in bladder size, leucocyte infiltration, tissue oedema, wall thickness, and intravesical lumen areas compared with the DMC group. Focally magnified images of the bladder wall showed that the DMC rats had scattered and shrunken bladder muscle, whereas the DM Li-ESWT rats had increased muscle volume, muscle bundle size, and muscle proportion. The parameters of the bladder wall were deteriorated in the DMC rats and partially reversed in the DM Li-ESWT rats. The DM Li-ESWT group had significantly smaller intravesical lumen areas (mean [SEM] 7.5 [0.7] mm² in the DMC group vs 3.3 [0.8] mm² in the DM Li-ESWT group; $P < 0.05$) and a higher proportion of muscle content (mean [SEM] 23.3 [1.1]% vs 28.4 [1.2]%).
in the DMC group vs 28.3 [1.1]% in the DM Li-ESWT group; \( P < 0.05 \). Furthermore, several additional histological findings in DM Li-ESWT group showed the improving trends but not in statistical significance, including lesser bladder cross-sectional area, bladder diameter, and bladder wall. Western Blot analysis revealed significantly reduced relative SMA expression of the bladder wall in DMC rats compared with NC and DM Li-ESWT rats, at a mean (SEM) of 100% in the NC group vs 68.7 (4.4)% in the DMC group vs 82.2 (4.2)% in the DM Li-ESWT group (\( P < 0.05 \)). Both the histological and Western Blot findings revealed restoration of the muscle content after Li-ESWT.

**Li-ESWT Enhances Bladder Nerve Regeneration**

Immunofluorescence microscopy with anti-S100 antibody staining under \( \times 400 \) magnification showed decreased nerve ending distribution and shortened nerve lengths in the bladder walls in DMC rats compared with NC and DM Li-ESWT rats (Fig. 6). Imaging at \( \times 1000 \) showed clear differences in the morphology of the nerve fibres amongst the groups: long and intact nerve fibres in the NC group; atrophied and fragmented nerve fibres in the DMC group; and regenerated nerve fibres in the DM Li-ESWT group. Quantification of the high-power images revealed significantly decreased nerve-ending distribution, at a mean (SEM) of 26.1 (1.2) in the NC group vs 13.9 (1.3) in the DMC group vs 26.9 (1.9) in the DM Li-ESWT group (\( P < 0.05 \)); and significantly shortened nerve length in DMC rats compared with NC and DM Li-ESWT rats, at a mean (SEM) of 23.8 (1.7) \( \mu m \) in the NC group vs 12.2 (1.5) \( \mu m \) in the DMC group vs 20.9 (1.3) \( \mu m \) in the DM Li-ESWT group (\( P < 0.05 \)). These data demonstrate that Li-ESWT promotes bladder nerve regeneration and innervation.

**Discussion**

UAB in the late stage of DBD is an intractable condition without effective treatment options [20,21]. The major purposes of the present study were to evaluate the therapeutic effect of Li-ESWT on a UAB rat model and to examine the feasibility of a reduced frequency, once-weekly Li-ESWT. Both of these domains showed promising results in acutely diabetic rats. The present study revealed that Li-ESWT...
improved bladder wall composition, activated bladder muscle regeneration, enhanced bladder and urethra muscle contractile function, improved bladder innervation, and promoted urethra continence. There are several strengths of the present study. First, the triad of conscious cystometry, LPP and \textit{ex vivo} organ-bath studies provided details of the functional changes in DM and the effects of Li-ESWT on the bladder and urethra. Second, the present study confirms the previous report of beneficial effect of the Li-ESWT on the diabetic bladder. Third, the positive effects in functional studies were further supported by results of immunohistology and Western Blot analysis.

On the contrary, there are a number of weakness and limitations of the present study. First, we conducted once-weekly Li-ESWT at a single energy level. Although we observed positive effects from this treatment regime, we cannot conclude that this is the optimal energy dosage for the once-weekly therapy. Elevating the energy level may increase the therapeutic benefit of ESWT, but also increase the risk of potential injury to the bladder. Further safety studies for Li-ESWT of the bladder should be conducted. Second, different UAB models may respond differently to Li-ESWT. Studies related to the effect of Li-ESWT on UAB are still sparse. Chuang et al. [22] reported that Li-ESWT improved voiding function and increased bladder contractile amplitude for myogenic detrusor underactivity in a cryoinjury UAB model. The present UAB model is DM-induced. Many factors may influence the induction of UAB, such as the degree of the hyperglycaemia and the duration of DM. Further studies of Li-ESWT on UAB in different animal models are needed. Third, we did not use markers to label stem cells in the present study. The therapeutic mechanism of ESWT related to stem cells has been previously investigated, including by our group [23,24]. In the present study, the potential effect of once-weekly Li-ESWT on bladder stem cells cannot be determined. All these weaknesses require further investigation.

In the present study, several parameters in conscious cystometry were improved after Li-ESWT. Lowering of the PVR is one of the most meaningful changes. UAB in the late stage of DBD causes high PVR and results in serious complications such as UTI, bladder stone formation, reflux hydronephrosis, and chronic renal insufficiency [20,21]. Much effort has been directed toward reducing the PVR; however,
few therapeutic modalities have been developed and the efficacies are still unsatisfactory. In the present study, we found that Li-ESWT reduces PVR by enhancing muscle contractile function, restoring bladder wall composition, promoting nerve regeneration, and ameliorating the coordination of bladder and urethra function. In the domains of the UI, our present study revealed an elevation in the LPP and an increase in urethral muscle contractile function in the organ-bath studies, which provided evidence for the therapeutic effect on UI by Li-ESWT. UI is a major complication of UAB in late-stage DBD. A large cohort study demonstrated that DM causes a three-fold higher risk of UI for ≥5 years [3]. The pathophysiology of UI includes diabetic neuropathy, microvascular damage, muscular dysfunction, and discoordination of the bladder and urethra [25]. The therapeutic mechanism of Li-ESWT involves angiogenesis, nerve innervation, tissue regeneration [26], and muscle function restoration [26,27]. Li-ESWT stimulates the regeneration of skeletal muscle and enhances tissue repair with the higher expression of the myonuclear content of regenerating muscle fibres [26]. Via the activation of protein kinase RNA-like endoplasmic reticulum kinase (PERK) and the transcription factor 4 (ATF4) pathway, Li-ESWT stimulates urethral muscle-derived stem cells, promotes myogenesis, and ameliorates UI [27]. Further investigations of the therapeutic effects and the mechanisms of Li-ESWT on UI are necessary.

Bladder remodelling comprises the morphological changes in the bladder wall due to polyuria and hyperglycaemic damage related to DM. The degree of alteration of each component of the bladder varies with the severity and duration of DM [28]. DM-related insulin resistance, metabolic syndrome, oxidative stress, chronic inflammation, and chronic ischaemia also play important roles in the progression of bladder remodelling [29]. In the present study, bladder enlargement, increased intravesical lumen area, increased bladder cross-sectional area, increased bladder diameter, increased bladder wall thickness, and decreased bladder smooth muscle content were all found in the DMC group. Li-ESWT partially reversed the morphological changes in the bladder, maintained the structure of the bladder, restored muscle volume, activated muscle regeneration, and decelerated the progression of DBD. Several studies on diabetic bladder remodelling have been performed and yielded inconsistent results [5,17,30]. These differences in the changes in the bladder wall, smooth muscle thickness...
Fig. 5 Bladder wall remodelling in DM and after Li-ESWT. (a) Representative microscopic images of the whole bladder for each group, stained with trichrome stain (×20). The DMC rats exhibited the pathological changes of UAB in late-stage DBD, including chronic inflammation, tissue oedema, and muscle atrophy of the bladder wall. The DM Li-ESWT group exhibited a decrease in bladder size, leucocyte infiltration, tissue oedema, wall thickness, and intravesical lumen areas compared with the DMC group. Magnified images (boxed regions) for each bladder are shown in the insets in panel b. (b) ×100 images of the bladder showed that the DMC rats had scattered and shrunken bladder muscle, whereas the DM Li-ESWT rats had an increase in muscle volume, muscle bundle size, and muscle proportion. (c) Bladder wall parameters were deteriorated in DMC and partially reversed in DM Li-ESWT. The DM Li-ESWT group had significantly smaller intravesical lumen areas and higher proportion of muscle content. (d) Western Blot analysis corroborates the above metrics, revealing the lowest relative SMA expression of the bladder wall in the DMC group compared with the NC and DM Li-ESWT groups, demonstrating restoration of the muscle content and activation of muscle regeneration after Li-ESWT. *P < 0.05 vs NC group; **P < 0.05 vs DMC group. Values are expressed as mean ± SEM.
and content amongst studies are likely due to dynamic changes of DBD and UAB associated with different severities and durations of DM in the different studies.

Regarding the weekly frequency of the regime for Li-ESWT, we reduced the frequency of ESWT to once weekly. The main purpose of this reduction was to have the better patient compliance, which has been well established to be highly correlated with convenience of the medical treatment [31]. A review of the association between dose regimen and medication compliance found that the daily prescribed dosage number is inversely correlated with compliance [32]; the lower the dosing frequency, the better compliance. Amongst the clinical applications of ESWT, most protocols use frequencies of two or three times per week, which is not only time- and cost-consuming for patients, but also reduces compliance [14,33]. A few studies have performed Li-ESWT once weekly and have reported promising outcomes [34,35]. ESWT is a dosage-dependent therapy [36]. We tried to balance the therapeutic effect and the weekly frequency of Li-ESWT to maintain both efficacy and compliance in patients. Here, we confirmed that reduced frequency, once weekly, Li-ESWT has a satisfactory therapeutic outcome in a UAB rat model. This provides the potential for the treatment of intractable UAB. Future studies should be performed to help translate the research finding into a possible clinical application for UAB.

Several mechanisms of ESWT have been proposed, including tissue regeneration, nerve re-innervation [37–39], angiogenesis [40], anti-inflammation [41], and stem cell activation and recruitment [23,24]. Vascular endothelial growth factor (VEGF) plays an important role in the angiogenesis effect of Li-ESWT. Combining with the effects from angiogenesis and anti-inflammation, studies reveal that Li-ESWT improves skin flap tissue survival and promotes wound healing by enhancing neovascularisation with increasing expression of VEGF and endothelial nitric oxide synthase (eNOS) and suppressing the inflammatory response by limiting leucocyte infiltration [40,41]. The enhancement of nerve re-innervation by ESWT is one of the key mechanisms of the therapeutic effect of ESWT on UAB. In the present study, immunofluorescence microscopic observations with anti-S100 antibody staining (a marker for Schwann cells of the

![Image](image_url)
peripheral nervous system, which has been used broadly for the identification of nerve innervation and nerve morphology [42]) revealed decreased nerve distribution and destroyed nerve structure caused by DM, which were found to be improved by Li-ESWT. Li-ESWT aids in re-innervation and therefore restoration of the nerve function in the bladder. The studies relating to Li-ESWT for the nerve regeneration have shown that Li-ESWT promotes nerve regeneration with increasing neuronal NOS (nNOS)-positive neurons, resulting in the amelioration of the erectile dysfunction [38]. The PERK/ATF4 pathway has been shown to enhance brain-derived neurotrophic factor expression in Schwann cells and promote nerve regeneration [39]. In addition, stem cell activation and recruitment also plays an important role in the mechanism of Li-ESWT. Li-ESWT stimulates the recruitment of endogenous progenitor cells [23], activates Schwann cells [24], induces the secretion and proliferation of bone marrow-derived mesenchymal stromal cells [43], and stimulates the expression of NGF and VEGF [17], resulting in angiogenesis, nerve regeneration, and tissue restoration.

In conclusion, once-weekly Li-ESWT ameliorated bladder dysfunction and UI in a diabetic UAB rat model. Li-ESWT improved bladder wall composition, activated bladder muscle regeneration, enhanced bladder and urethra muscle contractile function, increased bladder nerve innervation, and promoted urethra continence. Once-weekly Li-ESWT seems to have a positive effect on DM-induced UAB. Further investigations are warranted.

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Conflict of Interest

Tom F. Lue is a consultant for Acoustic Wave Cell Therapy, Inc.

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Abbreviations: ATF4, transcription factor 4; DBD, diabetic bladder dysfunction; DM(C), diabetes mellitus (control); EFS, electrical-field stimulation; Li-ESWT, low-intensity extracorporeal shockwave therapy; LPP, leak-point pressure; MCA, muscle contractile activity; NC, normal control; NGF, nerve growth factor; NOS, nitric oxide synthase; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PVR, post-void residual urine volume; SEM, standard error of the mean; SMA, smooth muscle actin; STZ, streptozotocin; UAB, underactive bladder; UI, urinary incontinence; VEGF, vascular endothelial growth factor.