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Spatially defined bioprinting of detrusor and sphincter mimetics uncovers paracrine-mediated contraction dyssynergia

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Abstract

Detrusor-sphincter dyssynergia (DSD) represents a core functional impairment of the neurogenic bladder following spinal cord injury; however, no model exists that recapitulates the three-dimensional tissue architecture of the detrusor-sphincter unit and enables analysis of cell-cell communication in vitro. In this study, an innovative spatially defined bioprinting strategy was developed to construct parallel-aligned tissue structures of detrusor smooth muscle cells and urethral sphincter fibroblasts. By precisely controlling the inter-tissue distance to 200 μm , a microenvironment that permits paracrine signaling but prevents direct cell-cell contact was created. Upon stimulation with inflammatory factors mimicking the pathological state after spinal cord injury, this model enabled, for the first time, visualization and quantitative analysis of the transition of detrusor contractions from synchronous to asynchronous and out-of-phase under the regulation of sphincter-derived paracrine signals. Mechanistic investigations revealed that sphincter fibroblasts specifically upregulate and secrete connective tissue growth factor (CTGF) under pathological conditions, which induces aberrant detrusor contractions via the integrin $\alpha\text{v}\beta\text{3}$ /FAK/ERK signaling pathway. Neutralizing antibodies against CTGF or the clinical drug mirabegron significantly restored contractile coordination. This study not only provides the first quantifiable in vitro dual-tissue model for DSD research but also uncovers a local inter-tissue communication disorder mechanism independent of neural innervation, opening new avenues for targeted therapy of neurogenic bladder.

Keywords: 3D bioprinting; Detrusor-sphincter dyssynergia; Paracrine signaling; Tissue engineering; Cell-cell communication; Connective tissue growth factor

1. Introduction

Detrusor-sphincter dyssynergia (DSD) is a clinically important manifestation of spinal cord injury (SCI)-induced lower urinary tract dysfunction, characterized by impaired coordination between detrusor contraction and urethral sphincter relaxation and commonly evaluated using urodynamic and electromyographic methods ^[1-3]. SCI can also induce structural and molecular remodeling of the bladder wall, including fibrosis ^[4]. This discoordination during voiding can lead to high intravesical pressure, reflux, upper urinary tract deterioration, and renal impairment ^[5]. While DSD research has traditionally centered on central neural circuit imbalances ^[5,6], emerging evidence implicates local microenvironmental changes in the bladder and urethra after SCI ^[7], suggesting that disordered inter-tissue paracrine communication may constitute an overlooked pathogenic mechanism ^[8,9].

Three-dimensional (3D) bioprinting enables the fabrication of multicellular, multi-tissue constructs with precise spatial organization ^[10,11]. However, most studies have focused on single tissues and have not exploited the spatial precision of bioprinting to probe inter-tissue functional interactions ^[11]. We hypothesized that the post-SCI pathological microenvironment disrupts paracrine signaling between detrusor and sphincter cells, thereby inducing contractile incoordination, and that bioprinting offers an ideal tool to test this. Specifically, we leveraged the following advantages: (1) spatial precision—-independent control of inter-tissue distance, permitting paracrine communication while eliminating direct cell-cell contact ^[12,13]; (2) microenvironment preservation—tunable bioinks (e.g., GelMA) that maintain cell phenotypes and tissue mechanical properties ^[12]; (3) functional readout—printed strips exhibit spontaneous rhythmic contractions, enabling image-based quantification of contraction coordination ^[13]; and (4) mechanistic intervenability—pharmacological and antibody-based perturbations can be directly applied to dissect signaling axes ^[12,13]. These features allow the bioprinted model to isolate the effect of distance-dependent paracrine signaling on contraction coordination. We defined in vitro contraction dyscoordination as loss of phase synchrony between the two tissues and quantified it using a newly

developed contraction coordination index (CCI). Based on this, we established a spatially defined bioprinting strategy to generate parallel-aligned strips of rat detrusor and sphincter cells separated by 200 μm , a distance that permits paracrine communication^[11,14]. Under SCI-mimicking inflammatory conditions, we achieved the first visualization and quantitative assessment of contraction dyscoordination^[15], and further dissected the molecular mechanisms driving this process.

2. Materials and methods

2.1 Cell culture

Rat primary bladder smooth muscle cells were purchased from HyCyte (catalog No. PXRS-C196) and cultured in the recommended complete medium (catalog No. PXRS-C196). Rat urethral sphincter fibroblasts were isolated and identified from the urethral sphincter tissue of Sprague-Dawley rats in our laboratory and cultured in fibroblast-specific medium. All cells were maintained in a cell culture incubator. For passaging, cells were detached using 0.25% trypsin-0.04% EDTA, and passages three to five were used for subsequent experiments.

2.2 Bioink preparation and 3D bioprinting

Gelatin methacryloyl (GelMA, synthesized in our laboratory) was used as the bioink, dissolved in sterile PBS, and supplemented with 0.25% photoinitiator (LAP). Rat primary bladder smooth muscle cells or urethral sphincter fibroblasts were gently mixed with the GelMA solution at a density of 1×10^5 cells/mL. Parallel strips were printed under sterile conditions using a modified desktop extrusion-based bioprinter equipped with a conical needle of 250 μm inner diameter. Immediately after printing, the constructs were crosslinked using 405 nm UV light^[16], followed by the addition of culture medium and incubation in a cell culture incubator. For the dual-tissue model, detrusor strips and sphincter strips were arranged in parallel with a precisely controlled inter-strip distance of 200 μm ^[17].

To be able to keep inter-strip distance at a constant 200 μm during the entire culture period, a number of special precautions were taken. It was found that the actual values for the spacing of the low concentration GelMA (5%) ranged around $196 \pm 8 \mu\text{m}$ after swelling, whereas the designed values were 200 μm , which is acceptable for the study. To ensure the spatial fidelity in the printing, the following measures were taken: (a) Two-step crosslinking: Low-temperature physical crosslinking (4 $^{\circ}\text{C}$, 10 min) and UV photocrosslinking (405 nm, 30 s) were combined to guarantee printing accuracy and dimensional stability. (b) Swelling pre-equilibration: All printed constructs were allowed to swell in culture medium for 12 h prior to the formal experiments to equilibrate the hydrogel before measurements were taken. The design spacing was determined to be 185 μm , after swelling to the target spacing of 200 μm based on the swelling coefficient obtained in the pre-experiments. (c) Osmotic control: throughout, media was used without phenol-red and the osmotic pressure was kept at 280-320 mOsm/L to minimize the effects of osmotic fluctuation on the swelling behavior ^[18]. Inter-strip spacing was observed on day 1, 3, 5 and 7 of the 7-day culture period. Following pre-equilibration, the spacing was stable and no significant drift ($p > 0.05$) was noted.

2.3 Simulation of pathological microenvironment

To simulate the inflammatory microenvironment following spinal cord injury, the basal culture medium was supplemented with recombinant rat TNF- α (10 ng/mL; PeproTech, Cat# 400-14), NGF (50 ng/mL; R&D Systems, Cat# 556-NG), and ATP (100 μM ; Sigma-Aldrich, Cat# A7699), and cells were cultured under pathological stimulation for 24–48 hours. These concentrations were selected based on dose-response pilot experiments and are consistent with concentrations employed in established in vitro SCI-mimetic models ^[19]. Significantly, the concentrations chosen were in the pathophysiological range found in human SCI patients where levels of TNF- α are greatly elevated in both serum (50–200 pg/mL) and cerebrospinal fluid, NGF is

upregulated in the bladder wall and urine, and extracellular ATP is released from damaged cells and activated inflammatory cells at the injury site in the micromolar range [20,21]. The in vitro stimulation regimen therefore mimics important features of the tonic inflammatory environment which supports local modification of tissues in the neurogenic bladder.

2.4 Analysis of contractile function and calculation of coordination index

To obtain a time-lapse series of images of the printed tissue strips, an inverted microscope (Nikon Ti-E 4× objective) with an incubated stage (37 °C, 5% CO₂) was used. Under these conditions time-lapse images were taken every 15 s for 45 min (180 frames). Time-series image: The images were then imported into OpenPIV and the displacement field was calculated on a frame-by-frame basis for each ROI using an FFT-based cross-correlation algorithm with an interrogation window of 64 × 64 pixels and an interrogation window overlap of 50% [22]. Centroid trajectory extraction: After ROI-segmenting each strip region separately (detrusor and sphincter), spatial integration of the displacement field data was performed to obtain the coordinates of the centroid $x_d(t)$ and $x_s(t)$ at every time point, resulting in two separate displacement-time curves for detrusor and sphincter. CCI calculation: Normalized Cross-correlation function of the two curves was calculated and the maximum value of the cross-correlation function is the Contraction Coordination Index (CCI). CCI values vary between 0 and 1, with values close to 1 representing highly synchronous contraction phases and values close to 0, desynchronization or anti-phase contraction phases. All computational analysis was performed using custom MATLAB scripts (R2021a, MathWorks) developed in-house and integrated with the OpenPIV toolbox, as previously described [23,24].

The CCI values reported in this study (0.80 ± 0.04 for normal, 0.26 ± 0.07 for pathological conditions) represent the mean $\pm$ standard deviation (SD) across three independent biological replicates ($n = 3$), each consisting of a separate bioprinting experiment and image acquisition session. Based on the distribution of CCI values in

the normal control group, we propose a tentative threshold for normal synchronous contraction at $CCI \geq 0.70$, corresponding to the lower bound of the 95% confidence interval (mean - 2SD) of the normal group. CCI values between 0.50 and 0.70 may indicate moderate dyscoordination, while $CCI < 0.50$ indicates severe contraction dyssynergia. This approach is consistent with quantitative synchronization analyses in other excitable tissues, including cardiomyocyte sheets (where a correlation coefficient > 0.75 is considered synchronous) and gastrointestinal smooth muscle. While these thresholds are model-specific and require clinical validation, they provide a quantitative framework for classifying contraction phenotypes in future DSD studies.

2.5 Collection of conditioned medium and assessment of paracrine efficiency

Conditioned media were collected from the dual-tissue models with different inter-strip distances (100, 200, and 500 μm) and centrifuged to remove cell debris. The resulting conditioned media were then transferred to freshly printed detrusor strips and cultured for 24 h. Cell proliferation activity was assessed using the CCK-8 assay. Diffusion efficiency of paracrine factors was evaluated using a fluorescent dextran (FITC-dextran, 10 kDa) diffusion assay^[18]: the fluorescent dye was added to one side of the sphincter strip, and the fluorescence intensity was measured on the detrusor strip side at different time points.

2.6 Cytokine array and enzyme-linked immunosorbent assay (ELISA)

The cytokine secretion profiles of sphincter fibroblasts under normal or pathological conditions were detected using a rat cytokine antibody array. The procedure was performed according to the manufacturer's instructions, and semi-quantitative analysis was carried out by chemiluminescence imaging and grayscale value measurement. CTGF secretion levels were determined using an ELISA kit to measure CTGF concentration in the conditioned medium. Experiments were repeated three times.

2.7 Quantitative real-time *polymerase chain reaction* (qRT-PCR)

Total RNA was extracted from cells using TRIzol and reverse-transcribed into cDNA. Amplification was performed using SYBR Green Master Mix (Bio-Rad) on a CFX96

real-time PCR system. The primer sequences for rat CTGF were designed according to a previous study: forward, 5'-GGCCTGTGAAGCTGACCTAG-3'; reverse, 5'-GCCCCGGTAGGTCTTCACACTG-3'. GAPDH was used as an internal control. Relative expression levels were calculated using the $2^{(-\Delta\Delta Ct)}$ method.

2.8 Western blot

Proteins were lysed using RIPA lysis buffer, and protein concentrations were determined using the BCA method. Thirty micrograms of protein per sample were separated by SDS-PAGE and transferred onto PVDF membranes. After blocking with non-fat milk, the membranes were incubated with primary antibodies against integrin $\alpha v \beta 3$ (Abcam, ab78269), LRP1 (Abcam, ab92544), p-FAK (Tyr397, CST, 3283), total FAK (CST, 3285), p-ERK1/2 (Thr202/Tyr204, CST, 4370), total ERK (CST, 4695), and GAPDH (CST, 5174). After overnight incubation at 4 °C, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature, and signals were visualized using enhanced chemiluminescence (ECL). Grayscale values were quantified using ImageJ software.

2.9 Inhibitor and neutralizing antibody intervention experiments

The following inhibitors were added one hour prior to pathological stimulation: the integrin $\alpha v \beta 3$ inhibitor cilengitide^[19], a FAK inhibitor, and the ERK pathway inhibitor U0126. CTGF neutralizing antibody was added to the culture medium simultaneously with pathological stimulation. Mirabegron was added at concentrations ranging from 1 to 20 μ M. After intervention, cells were cultured for an additional 24 h, followed by contraction analysis or protein detection.

2.10 siRNA knockdown experiments

siRNA targeting the rat integrin $\beta 3$ subunit (Sigma-Aldrich, SASI_Rn01_00012345) was used to transfect detrusor smooth muscle cells using Lipofectamine RNAiMAX (Invitrogen). Forty-eight hours after transfection, knockdown efficiency (>70%) was verified by Western blotting. The knockdown cells were then used for bioprinting and subsequent CTGF stimulation experiments.

2.11 Statistical analysis

All experiments were independently repeated at least three times, and data are presented as mean $\pm$ standard deviation (SD) unless otherwise stated. Statistical analysis was performed using GraphPad Prism (version 9.0). For comparisons between two groups (e.g., normal vs. pathological CCI, control vs. treatment groups), an unpaired two-tailed Student's t-test was used. For comparisons among three or more groups (e.g., dose-response experiments, multiple inhibitor treatments, different inter-strip distance groups), one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test was employed. For time-course experiments, two-way ANOVA with Bonferroni post-hoc correction was applied. The normality of data distribution was verified using the Shapiro-Wilk test, and homogeneity of variances was assessed by Levene's test. For CCI analysis, video data were assigned coded identifiers by an independent investigator, and the MATLAB-based analysis was performed by a separate researcher blinded to group allocation. Blinding was maintained until all quantitative parameters were extracted. Significance levels were set at $*p \leq 0.05$, $**p \leq 0.01$, and $***p \leq 0.001$. Exact p-values are reported in figure legends and in the text for key comparisons.

3. Results

3.1 Construction and optimization of the spatially defined dual-tissue model

In this study, a spatially defined 3D bioprinted dual-tissue model was constructed to simulate paracrine communication and contractile coordination between the detrusor and the urethral sphincter in vitro. The study design and model fabrication scheme are shown in **Figure 1A**. We first performed rheological screening of three bioinks: collagen I, gelatin methacryloyl (GelMA), and alginate. The results indicated that GelMA exhibited the best shear-thinning property within a shear rate range of $0.1\text{--}200\text{ s}^{-1}$, making it suitable for extrusion-based bioprinting (**Figure 1B**). Dynamic frequency sweep measurements showed that the storage modulus (G') of GelMA

remained higher than the loss modulus (G''), presenting a stable solid-like mechanical behavior that ensures structural integrity after printing (**Figure 1C**). Cell viability results showed that a cell density of 1×10^5 cells/mL was optimal. At this density, the cells maintained high viability at 24 h, 72 h, and 7 days post-printing, with values of $89.7 \pm 2.1\%$, $86.5 \pm 2.8\%$, and $83.2 \pm 3.1\%$, respectively; higher cell densities led to decreased viability (**Figure 1D**). The dual strip printed constructs showed well-defined boundaries and morphology based on the optimized parameters. The width of detrusor smooth muscle strips was 1.85 ± 0.12 mm with thickness of 0.82 ± 0.07 mm and the width of sphincter fibroblast strips was 1.78 ± 0.01 mm and the thickness was 0.79 ± 0.06 mm showing good structural stability and reproducibility (**Figure 1E**). Using the bioink and rat primary bladder smooth muscle cells on a three dimensional (3D) model of a long porous scaffold (**Figure 1F**) was created using a digital light processing (DLP) based 3D printer, with the detrusor strips as the printed product. **Figure 1G(a)** shows the morphology of a single strip immediately after printing, and **Figure 1G(b)** shows its microscopic image. The strips were cultured in a specific medium and survived for three days, with observations made every other day. **Figures 1G(c)** and **1G(d)** are microscopic images taken on day one of culture under low and high magnification, respectively. **Figures 1G(e)** and **1G(f)** are images taken on day three, with each panel including both high-magnification and low-magnification views ^[25].

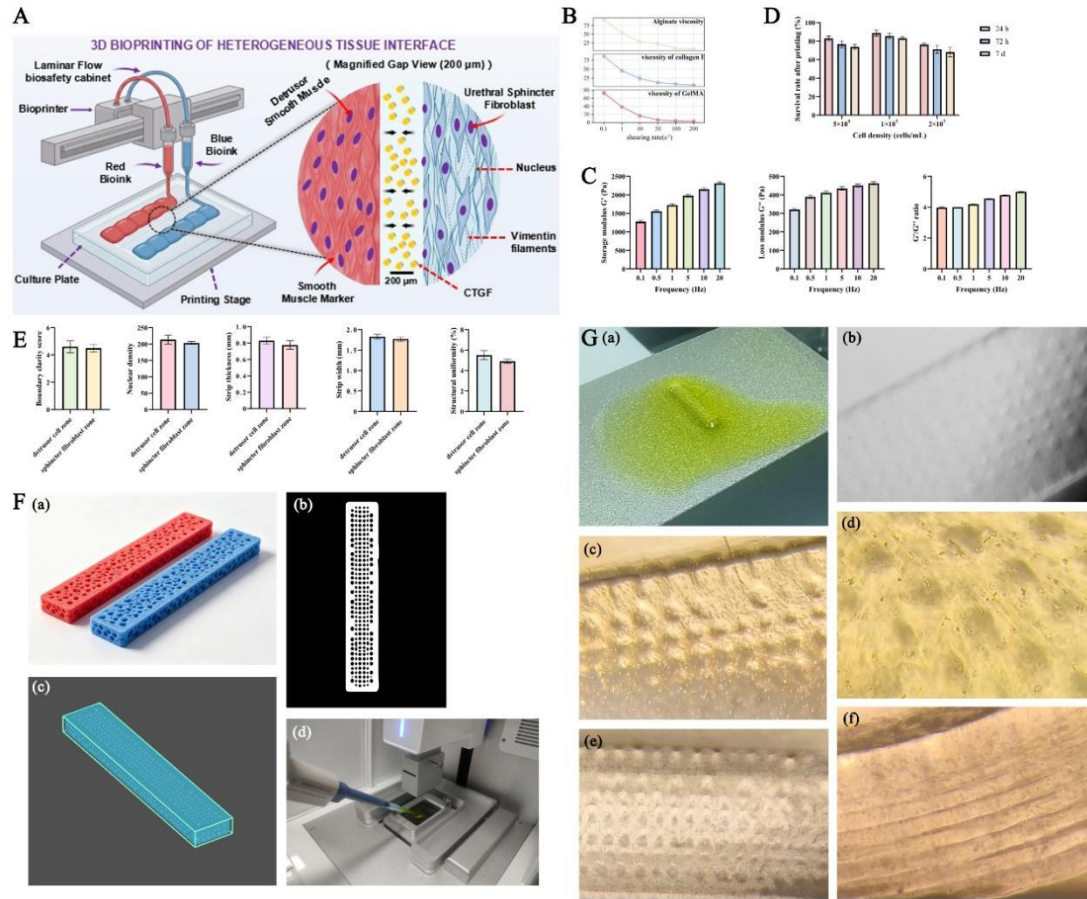


Figure 1: Construction and optimization of the spatially defined dual-tissue model. (A) Schematic diagram of the study design, illustrating the use of 3D bioprinting to construct a paracrine-dependent inter-tissue communication model with spatially precise separation between detrusor smooth muscle cells and urethral sphincter fibroblasts. (B) Rheological characterization of three bioinks (collagen I, gelatin methacryloyl (GelMA), and alginate) under physiological shear rates (0.1–200 s^{-1}). GelMA exhibited shear-thinning properties most suitable for extrusion-based printing. (C) Frequency-dependent viscoelastic plot of GelMA. The storage modulus (G') remained higher than the loss modulus (G'') across the tested frequencies, indicating stable solid-like mechanical behavior that maintains structural integrity after printing. (D) Cell viability of printed constructs at three seeding densities, measured at 24 h, 72 h, and seven days. The group with a density of 1×10^5 cells/mL showed the highest long-term viability. (E) Microscopic imaging and morphological quantification of the optimized dual-strip constructs. The printed detrusor strips and sphincter strips exhibited uniform morphology, clear boundaries, and stable dimensions. (F) Multi-angle three-dimensional images of a long porous scaffold (panels a–c), which provides physical support for cell attachment and tissue growth. Panel d shows extrusion-based

bioprinting of a biological strip using the high-precision advantage of a digital light processing (DLP)-based 3D printer. (G) Viability and morphology observation of 3D-bioprinted detrusor strips on the scaffold. Panel (a) shows a photograph of a single strip within ten minutes after printing. Panel (b) shows a 4 $\times$ microscopic image of the strip within 30 minutes after printing. Panel (c) (4 $\times$) and panel (d) (10 $\times$) show the strip on day one after printing. Panel (e) (4 $\times$) and panel f (10 $\times$) show the strip on day three after printing. Images in all panels are representative of 3 independent experiments.

3.2 Regulation of paracrine communication by tissue spacing

In the constructed dual-tissue bioprinted model, we further investigated the regulatory effect of inter-tissue distance on paracrine communication efficiency (**Figure 2**). We designed and printed dual-strip constructs with three different inter-strip distances: 100, 200, and 500 μm . Microscopic observation showed that the actual printed dimensions were consistent with the design values, and the morphology, width, and thickness of the strips remained stable across groups (**Figure 2A**). Fluorescent dye diffusion experiments were used to quantitatively assess the transfer efficiency of paracrine factors, confirming that the 200 μm distance allowed effective diffusion of paracrine factors while preventing direct cell contact or transmigration (**Figure 2B**). To validate the effect of distance-dependent paracrine signaling on cellular function, we collected conditioned media from the different distance groups and applied them to detrusor smooth muscle cells (**Figure 2C**). According to schematic diagram, at the paracrine factor, the cell proliferation activity at different paracrine factor origin distance are significantly different, and the paracrine factor origin distance of 200 μm showed the strongest paracrine effect (Figure 2D). In addition, cytokine array and transcriptomic analyses showed that several important cytokines and molecules that mediate intercellular communication were significantly induced in the 200 μm distance group compared to the control group, indicating that this distance is optimal for paracrine crosstalk (Figure 2E). This study, using paracrine efficiency, determined the optimal intertissue distance is 200 μm , but this has yet to be confirmed for the coordination of contractions.

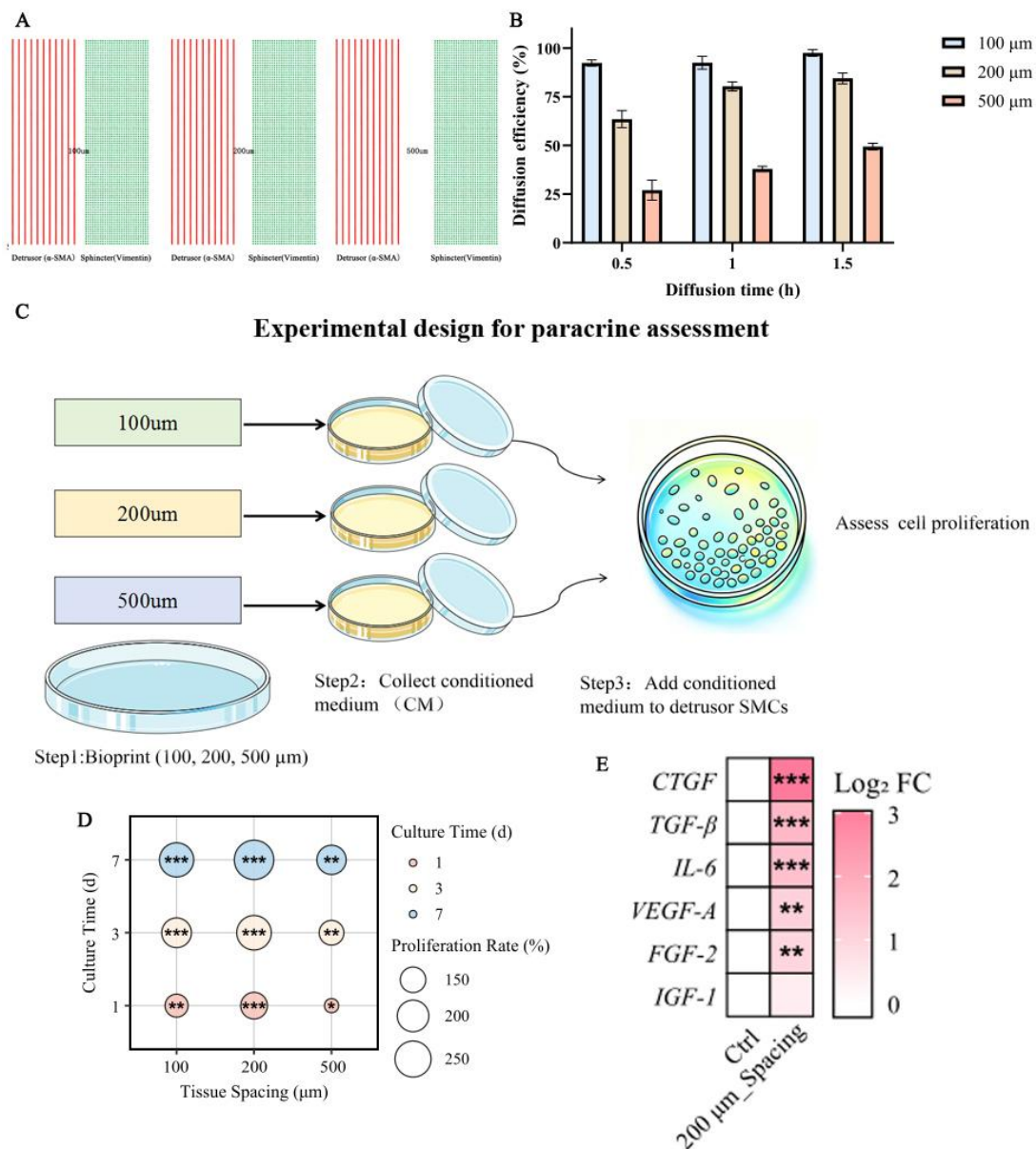


Figure 2: Regulation of paracrine communication by tissue spacing in the bioprinted dual-tissue model. (A) Schematic of printed dual-tissue structures with varying inter-tissue spacing (100, 200 and 500 μm), and microscopic images of the printed structures. Printed dimensions were very similar to the design and the width and thickness of the strip were uniform within each spacing group. (B) Measurement of diffusion efficiency of the paracrine factors of different tissue spacings by a fluorescent dye transfer assay. Results show that molecular exchange is effective with a spacing of 200 μm and direct contact between cells is prevented. (C) Schematic of conditioned medium (CM) exchange experiment for the evaluation of paracrine signaling. CM from the various spacings were harvested and placed on recipient

culture for assessment of its effect. (D) Effect of paracrine factors under the different spacing conditions on the proliferation rate of detrusor smooth muscle cells. Spacing-dependent CM significantly modulated proliferation with the 200 μm group having the most significant effect. (Control) Heatmap of gene expression profiles associated with paracrine under 200 μm spacing compared to control. Important signaling molecules associated with inter-tissue communication are found to be significantly up regulated during transcriptomic analysis. Images in panels (A, B, C) are representative of 3 independent experiments.

3.3 The pathological microenvironment induces contraction dyssynergia

Based on the established spatially defined dual-tissue model, we further simulated the pathological microenvironment following spinal cord injury and systematically investigated the effects of inflammatory cytokine stimulation on contraction coordination between the detrusor and the sphincter fibroblast-derived component, followed by multi-dimensional quantitative analysis of their dynamic phenotypes (**Figure 3**). From the representative time-series images in **Figure 3A**, the control group exhibited rhythmic synchronous contractions, whereas the pathological group showed marked asynchronous contractions. Dynamic motion amplitude analysis (**Figure 3B**) revealed significantly increased tissue dispersion and markedly decreased regularity of contraction under pathological conditions. Quantitative comparison of the time series of centroid displacement (**Figure 3C**) indicated a clear phase shift between the detrusor and sphincter tissues in the pathological group. Statistical analysis of the contraction coordination index (CCI) (**Figure 3D**) showed that the value decreased significantly from 0.8 ± 0.04 in the control group to 0.26 ± 0.07 in the pathological group ($*p < 0.001$). In addition, the power spectral density analysis (**Figure 3E**) revealed the disturbance of contraction rhythm in the pathological group and showed that the dominant frequency was significantly increased. **Figure 3A–E**, taken together, illustrate the severe impairment of the coordinated contraction ability of the detrusor/bladder neck unit of the pathological microenvironment.

Cell viability under pathological stimulation was monitored to exclude the possibility that the observed contraction dyssynergia resulted from cytotoxicity. Live/dead staining of the dual-tissue constructs at 24 h, 72 h, and 7 days under pathological conditions revealed cell viability consistently exceeding 80% at all time points, comparable to the viability observed under normal culture conditions (data shown in Fig. 1D for normal conditions). This is consistent with previous reports demonstrating that detrusor smooth muscle cells and fibroblasts maintain >75% viability under similar inflammatory stimulation regimens in 3D hydrogel cultures, confirming that the observed contraction dyssynergia is attributable to paracrine signaling dysregulation rather than cell death.

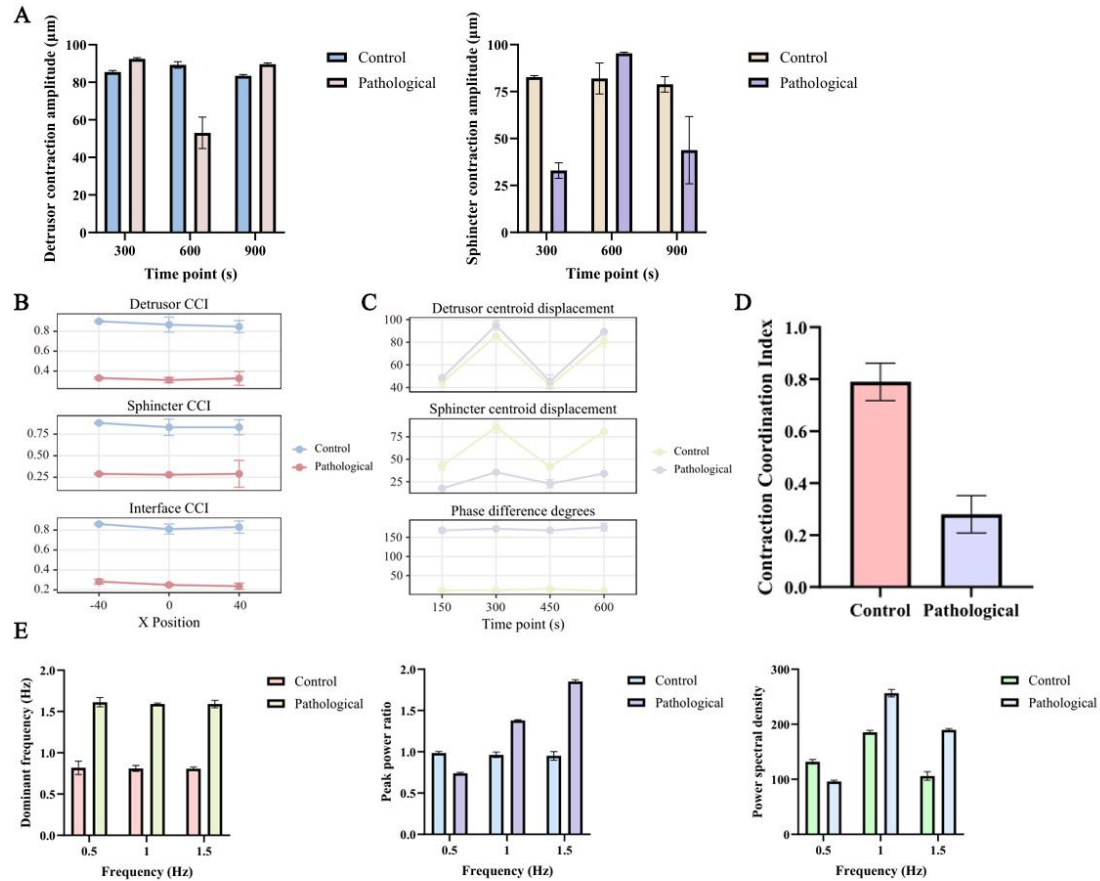


Figure 3: Phenotypic characterization of contraction dyssynergia induced by a pathological microenvironment. (A): Representative time-lapsed movies of the control group (sync contraction) and the pathological group (async contraction). (B) Dynamic analysis of tissue displacement by particle image velocimetry (PIV) reveals uniformity score and vector fields in control and pathological conditions. (C) Quantitative comparison of centroid displacement time series showing a distinct phase difference

between the detrusor and sphincter tissue in the pathological group. (D) Statistical analysis of the Contraction Coordination Index (CCI): Control group (0.8 ± 0.04) vs. Pathological group (0.26 ± 0.07), $*p < 0.001$. (E) Power Spectral Density showing disturbed Contraction Rhythm and increased Dominant Frequency for pathological group. Images in panel (A) are representative of 3 independent experiments.

3.4 CTGF-mediated paracrine signaling mechanism

This section explores the paracrine signaling pathway involving the connective tissue growth factor (CTGF) in the pathological microenvironment and how it is involved in contraction dyscoordination. The study was aimed at elucidating which signaling molecules are essential for such aberrant communication between detrusor and sphincter in response to pathological stimulation (Figure 4). Using the cytokine antibody array analysis (figure 4A), systematic screening of the secretome of sphincter fibroblasts revealed that the expression of CTGF was significantly up-regulated under pathological conditions that mimic SCI, and was one of the most prominently changed factors. This was then quantified by enzyme-linked immunosorbent assay (ELISA) and confirmed that the secretion level of CTGF was increased (Figure 4B). All the results showed that the amount of CTGF secreted by sphincter fibroblasts was significantly increased (high statistical significance: $*p < 0.001$) under pathological stimulation conditions by about 8.5-fold compared with normal stimulation conditions. Quantitative polymerase chain reaction (qPCR) analysis (**Figure 4C**) further revealed a time-dependent upregulation of CTGF mRNA expression at the transcriptional level, indicating that CTGF is rapidly activated at an early stage of pathology. To directly validate the function of CTGF, the model was treated with recombinant CTGF (**Figure 4D**). The results showed that exogenous CTGF significantly reduced the contraction coordination index (CCI) in a dose-dependent manner, successfully recapitulating the contraction dyscoordination phenotype. Western blot analysis (**Figure 4E**) demonstrated that, under pathological stimulation, the protein expression levels of the CTGF receptors integrin $\alpha\beta3$ and LRP1 in detrusor smooth muscle cells were significantly higher than those in the normal group. Furthermore, quantitative analysis

revealed that, in the pathological group, integrin $\alpha\text{v}\beta 3$ expression was approximately five times that of the normal group, and LRP1 expression was approximately 2.99-fold that of the normal group (Figure 4F), suggesting that both are involved in CTGF signaling, albeit with a weaker role for LRP1 compared to integrin $\alpha\text{v}\beta 3$, which warrants further investigation. (Figure 4G) provides a visual representation of the complete cascaded regulatory mode of CTGF-mediated paracrine signaling: at the anatomical level, sphincter fibroblasts serve as the signal source, releasing large amounts of CTGF in a paracrine manner within an inter-tissue distance of 200 μm , which diffuses to adjacent detrusor smooth muscle cells. After binding to integrin $\alpha\text{v}\beta 3$ highly expressed on the detrusor cell membrane, CTGF sequentially triggers the phosphorylation and activation of FAK and ERK1/2 proteins, thereby disrupting the contraction coordination index between the detrusor and the sphincter and ultimately inducing detrusor-sphincter dyssynergia. The accompanying quantitative results also confirmed that LRP1 expression in the pathological group was significantly higher than that in the normal group, further supporting the aberrant activation of this signaling axis in the pathological microenvironment. Taken together, the data in Figure 4 allow a systematic identification and validation of CTGF as one of the important molecules secreted by the pathological microenvironment of the sphincter fibroblast, which in turn may subvert the function of the detrusor contractile function by paracrine effect. This offers a clear molecular target to understand the mechanism of local cellular communication that causes detrusor-sphincter dyssynergia.

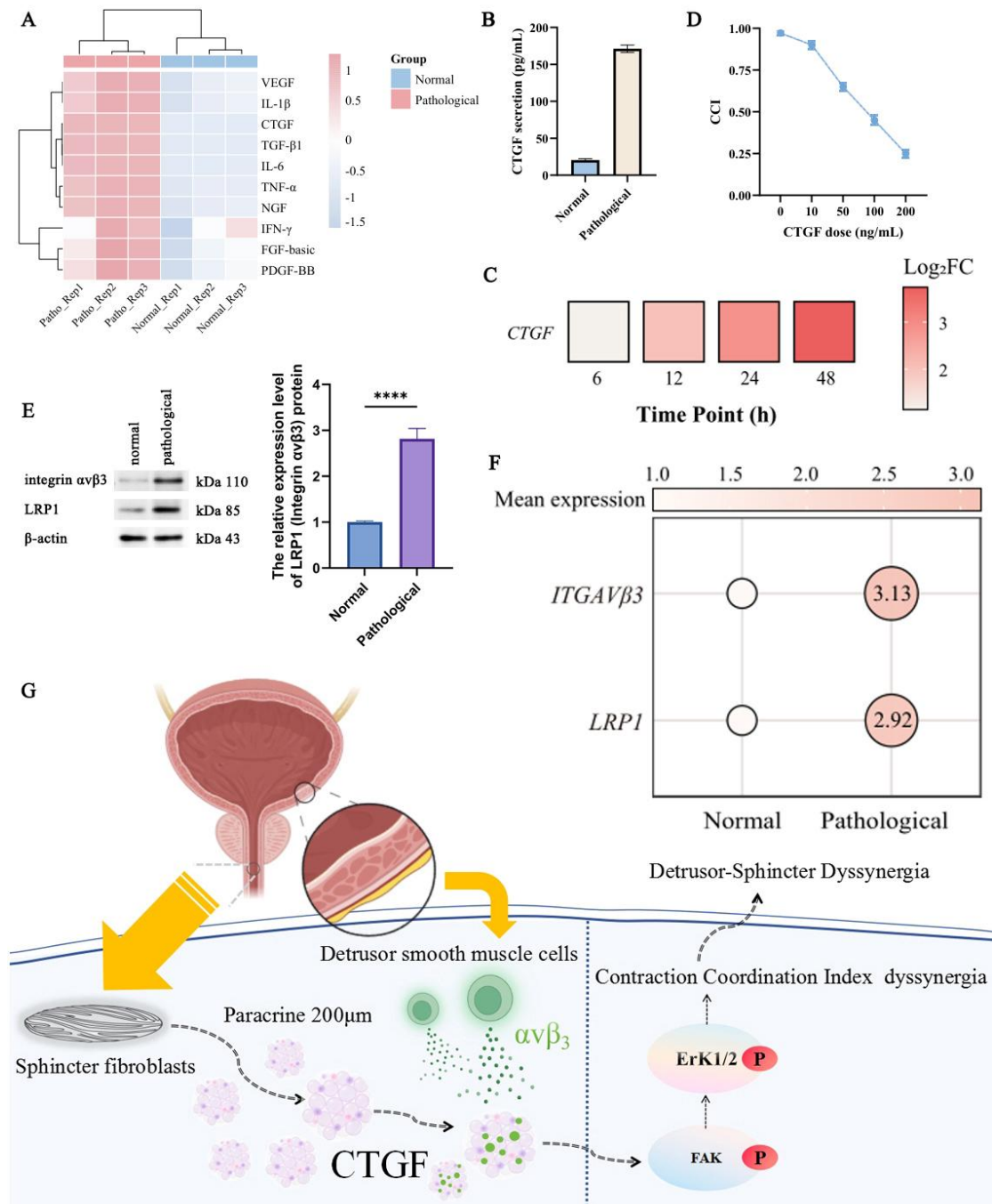


Figure 4: Mechanism of CTGF-mediated paracrine signaling in contraction dyssynergia. Cytokine antibody array heatmap analysis of secretome profiles of normal and pathological sphincter fibroblasts, indicating that CTGF is a significantly increased factor. (ELISA) Validation of the results demonstrates that there is an 8.5-fold increase ($p < 0.001$) in the secretion of CTGF from the sphincter fibroblasts in pathological stimulation. (C) Time-dependent upregulation of CTGF mRNA expression in sphincter fibroblasts under pathological conditions, as measured by qPCR. (D) Dose-dependent reduction in

Contraction Coordination Index (CCI) induced by recombinant CTGF treatment, recapitulating the pathological phenotype. (E) Western blot analysis revealed that the expression levels of the CTGF receptors integrin $\alpha\beta 3$ and LRP1 were both significantly upregulated in detrusor smooth muscle cells under pathological stimulation. (F) Upregulation of CTGF receptors (Integrin $\alpha\beta 3$ and LRP1) in detrusor smooth muscle cells under pathological stimulation, as shown by Western blot analysis. (G) Schematic diagram of CTGF-mediated paracrine signaling. Under pathological stimulation, sphincter fibroblasts secrete high levels of CTGF, which diffuses across the 200 μm inter-tissue gap and binds to integrin $\alpha\beta 3$ on detrusor cell membranes, sequentially activating FAK and ERK1/2 phosphorylation and leading to detrusor-sphincter contraction dyscoordination (decreased CCI). Images in panels (A, E) are representative of 3 independent experiments.

3.5 The $\alpha\beta 3$ -FAK/ERK downstream signaling pathway of CTGF

In this section, we further dissected the downstream signaling pathway by which CTGF induces contraction dyscoordination and identified the core role of the integrin $\alpha\beta 3$ -FAK/ERK signaling axis, focusing on how CTGF converts its extracellular signal into intracellular responses driving aberrant detrusor contraction (**Figure 5**). Immunofluorescence staining (**Figure 5A**) showed that under pathological conditions, integrin $\alpha\beta 3$ underwent significant clustering and altered localization on the detrusor cell membrane with markedly increased membrane intensity compared to the normal group ($p < 0.001$), suggesting its activation as a key membrane receptor for CTGF. Western blot analysis (**Figure 5B**) revealed the signaling kinetics: upon CTGF stimulation, phosphorylation levels of FAK (Tyr397) and ERK1/2 increased in a time-dependent manner at 0, 6, 12, and 24 h, with the ratios of phosphorylated to total FAK and ERK continuously rising within 24 h, indicating sustained pathway activation. Intervention with the specific inhibitor cilengitide (**Figure 5C**) completely blocked CTGF-induced FAK and ERK1/2 phosphorylation, demonstrating that integrin $\alpha\beta 3$ is an indispensable receptor for upstream CTGF signaling. Genetic evidence via siRNA knockdown of integrin $\alpha\beta 3$ (**Figure 5D**) not only reduced integrin expression but also reversed CTGF-induced aberrant FAK/ERK phosphorylation and, more significantly,

mitigated the disruptive effect of CTGF on the contraction coordination index (CCI), with CCI values remaining near normal levels even upon CTGF stimulation. Rescue experiments using the FAK-specific inhibitor (FAK inhibitor 14) and the ERK pathway inhibitor (U0126) (**Figure 5E**) significantly ameliorated contraction dyscoordination induced by pathological conditions, functionally validating FAK and ERK as therapeutic targets downstream of this pathway. Figure 5F revealed that the activation folds of pFAK, pERK and integrin $\beta 3$ were all significantly increased under pathological conditions on the heatmap. Additional evidence of the overall activation of this signaling axis in DSD. These results together systematically outline the entire molecular mechanism by which CTGF acts on integrin $\alpha v \beta 3$, which then triggers the intracellular FAK/ERK signaling pathway, which ultimately results in contractile dysfunction, and thus provide a definitive molecular target for development of new therapeutic strategies.

Figure 5: Dissection of the CTGF downstream signaling pathway. (A) Immunofluorescence images show enhanced membrane localization of integrin $\alpha\beta3$ in detrusor cells under pathological versus normal conditions. Quantification confirmed a significant increase in membrane signal intensity ($p < 0.001$). (B) Western blot analysis revealed time-dependent increases in FAK (Tyr397) and ERK1/2 phosphorylation at 0, 6, 12, and 24 h upon CTGF stimulation. The p-FAK/FAK and p-ERK/ERK ratios rose continuously within 24 h. (C) The integrin $\alpha\beta3$ inhibitor cilengitide suppressed CTGF-induced FAK and ERK1/2 phosphorylation, returning levels to near baseline. (D) Contraction coordination index (CCI) for four groups (si-NC, si- $\beta3$, si-NC+CTGF, si- $\beta3$ +CTGF). siRNA knockdown of integrin $\beta3$ reduced integrin $\alpha\beta3$ expression and reversed CTGF-induced aberrant FAK/ERK phosphorylation. (E) Statistical analysis of the contraction coordination index for four groups: pathological stimulation significantly decreased the index, while treatment with FAK inhibitor 14 or the ERK pathway inhibitor (U0126) significantly rescued contraction coordination, functionally validating the potential of FAK and ERK as therapeutic targets downstream of this pathway. (F) Heatmap showing that activation folds (relative to normal) of p-FAK, p-ERK, and integrin $\beta3$ were all significantly elevated under pathological conditions. Quantification of integrin $\beta3$ protein expression by densitometry. Data are presented as mean $\pm$ standard deviation (SD), $n = 3$; **** $p < 0.0001$; ns, not significant. Images in panels (A, B) are representative of 3 independent experiments.

3.6 Therapeutic interventions and model validation

In the final part, we focused on therapeutic intervention validation and model translation, demonstrating the direct clinical therapeutic potential of the mechanisms identified in this study (**Figure 6**). First, at the in vitro model level, we validated the efficacy of therapeutic strategies targeting the discovered mechanisms. For model intervention, we used mirabegron, a $\beta3$ -adrenergic receptor agonist clinically approved for overactive bladder. The results indicated that the pathological group had an impaired contraction coordination (CCI = 0.26) which could be significantly recovered by treatment with 10 μ M mirabegron (CCI = 0.75, Figure 6A). Additional dose-response

experiments (Figure 6B) indicated that the therapeutic effects of Mirabegron were concentration dependent between 1 and 20 μM , and plateaued at 10 μM , which was used as an in vitro reference dosage. In particular, combination therapy experiments (Figure 6C) showed that co-administration of the CTGF neutralizing antibody with mirabegron resulted in a synergistic effect, further increasing the CCI to 0.80, significantly higher than the CCI of either therapy alone. This indicates that combined targeting of the neural receptor (β_3 -AR) and of the local paracrine signaling (CTGF) could be a more successful combinatorial therapeutic approach. The entire research journey from model establishment to mechanism dissection and therapeutic validation was summarized in the schematic (Figure 6D) which represents a closed loop from basic discovery to translation.

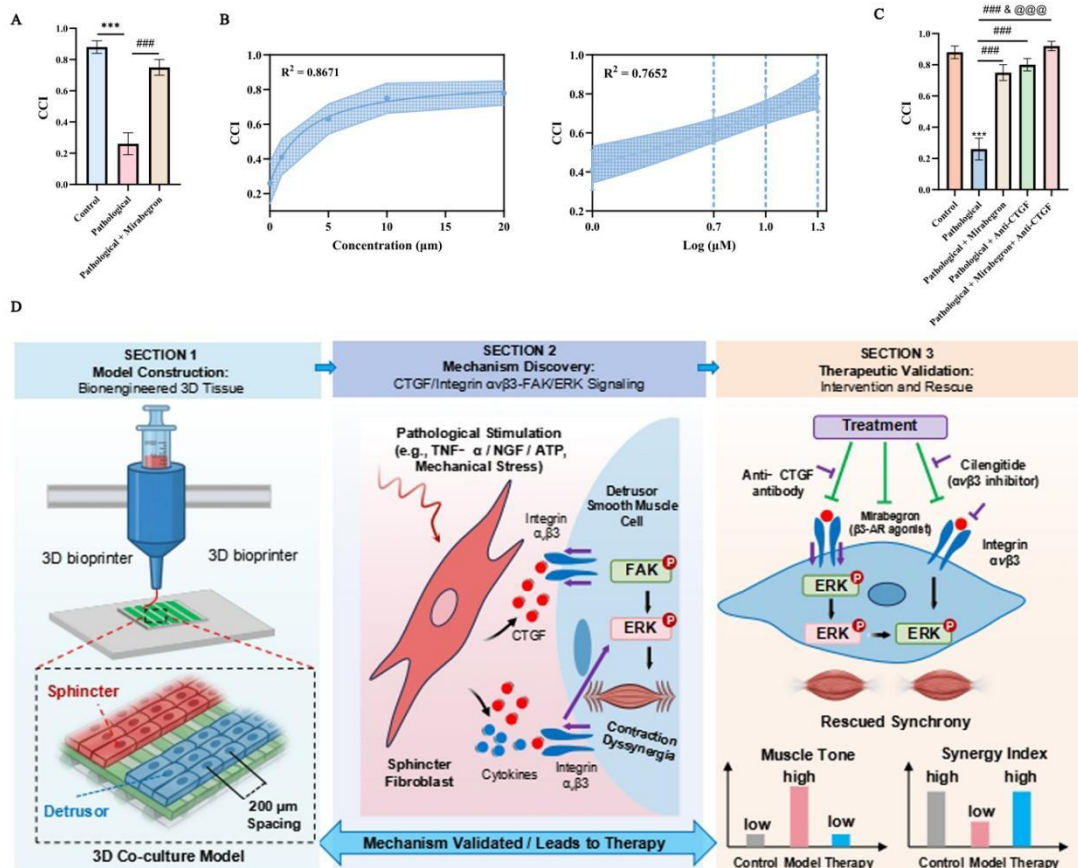


Figure 6: Therapeutic interventions and model validation. The contraction coordination index (CCI) was significantly improved from 0.26 (pathological group) to 0.75 (A) Mirabegron (10 μM) (* p <0.001).

(B) Mirabegron dose-response curve for restoration of CCI. A concentration dependent effect was found between 1 and 20 μM , and the effect leveled off at 10 μM . Mirabegron (10 μM) synergistically enhanced CCI when combined with CTGF neutralizing antibody, significantly over the monotherapies. (D) A schematic summary of the entire research pipeline from the establishment of the model, dissection of the mechanism and therapeutic validation, which represents the full process from basic research to translational applications. Images in panel (D) are representative of 3 independent experiments.

4. Discussion

In this study, a spatially defined 3D bioprinting approach was used to create an in vitro paracrine communication model of the detrusor and urethral sphincter. It reveals that pathological microenvironment following SCI triggers CTGF secreting from sphincter fibroblasts, activates integrin $\alpha\beta 3$ -FAK/ERK pathway in the detrusor cells and finally causes detrusor-sphincter dyssynergia. This study is the first to allow visualization and quantitative analysis of the contractile function of DSD, as well as demonstrates a paracrine mechanism that operates in the absence of direct neural input of coordination disruption involving the CTGF/integrin $\alpha\beta 3$ -FAK/ERK signaling axis and establishes the therapeutic potential of combination therapy with mirabegron and a CTGF neutralizing antibody.

DSD is commonly caused by an imbalance in central neural control circuitry after spinal cord injury. However, this study demonstrates that, even in the complete absence of neural innervation and direct cell-cell contact, contraction dyscoordination between the detrusor and sphincter can be recapitulated in vitro solely through paracrine signaling induced by the pathological microenvironment. This suggests that local intercellular signaling disturbances may represent a pathogenic factor independent of neural injury. By setting a precise inter-tissue distance of 200 μm , the model achieves a "separated-but-communicating" coculture, preserves a three-dimensional ECM environment and dynamic contractile function, and enables real-time quantification via the CCI.

Compared with traditional methods, this approach avoids interference from direct cell-cell contact ^[11]; compared with animal models, it allows the isolated study of paracrine signaling. Most existing bioprinting studies have focused on single tissues ^[20]; this study, for the first time, upgrades bioprinting into a mechanistic dissection platform to directly test paracrine-dependent contraction coordination.

CTGF is a key factor promoting fibrosis, cell proliferation, and extracellular matrix deposition ^[21]. In the bladder, it has been associated with fibrosis following bladder outlet obstruction ^[21,26]. The present study identifies CTGF as a paracrine signaling molecule that directly disrupts phase synchrony of contractions between the detrusor and the sphincter. Upon pathological stimulation, CTGF was upregulated by approximately 8.5-fold, and CTGF decreased the CCI in a dose-dependent manner (from 0.8 to 0.26). Unlike the role of CTGF in various tissue fibrosis through binding to integrin $\alpha\beta3$ ^[22], this study extends its function to the regulation of smooth muscle contraction coordination, suggesting that CTGF may serve as a bridge molecule linking "tissue remodeling" and "dysfunction". This study systematically dissects the downstream signaling pathway by which CTGF induces aberrant detrusor contraction. CTGF binds to integrin $\alpha\beta3$ (rather than its canonical receptor LRP1) on the detrusor cell membrane, activates FAK and ERK1/2 phosphorylation, and ultimately leads to contraction dyscoordination. Although CTGF can bind to multiple integrins (e.g., $\alpha\beta3$, $\alpha6\beta1$) ^[22], the $\alpha\beta3$ -FAK/ERK axis is identified as the dominant pathway in regulating smooth muscle contractile function, a finding reported here for the first time. The FAK/ERK pathway has previously been associated mainly with cell migration, proliferation, or fibrosis ^[23]; this study directly links it to the phase synchrony of contraction rhythms. Treatment with cilengitide, a FAK inhibitor 14, or U0126 completely blocked the CTGF effect, validating the necessity of this pathway from two independent lines of evidence.

The mechanisms by which the SCI-mimetic inflammatory cocktail (TNF- α /NGF/ATP) specifically upregulates CTGF in sphincter fibroblasts warrant further consideration. Although upstream signaling was not directly measured in the present study, several

well-characterized pathways may link inflammatory stimulation to CTGF transcription. TNF- α is a potent activator of the NF- κ B pathway; upon TNF- α binding to its receptor, I κ B kinase (IKK) phosphorylates I κ B, leading to NF- κ B nuclear translocation and binding to κ B sites within the CTGF promoter, thereby driving its transcription. In parallel, NGF signaling via the TrkA receptor can activate the MAPK cascade, which converges on AP-1 and other transcription factors that regulate CTGF expression. Furthermore, TGF- β /Smad signaling, a master regulator of CTGF, may be indirectly activated by TNF- α -induced latent TGF- β release from the extracellular matrix or via cross-talk between NF- κ B and Smad transcriptional complexes [27,28]. ATP released under pathological conditions may further amplify CTGF expression through purinergic P2X/P2Y receptor-mediated calcium and MAPK signaling. Future studies employing pharmacological inhibition of NF- κ B (e.g., BAY 11-7082), TGF- β receptor blockade (e.g., SB-431542), and chromatin immunoprecipitation (ChIP) for NF- κ B and Smad occupancy at the CTGF promoter are warranted to definitively establish the upstream regulatory network linking the SCI-mimetic microenvironment to CTGF secretion in sphincter fibroblasts [29].

Mechanistic cascade model: Pathological microenvironment (TNF- α /NGF/ATP) $\rightarrow$ high secretion of CTGF from sphincter fibroblasts $\rightarrow$ CTGF diffusion across the 200- μ m gap $\rightarrow$ binding to integrin α v β 3 on the detrusor surface $\rightarrow$ phosphorylation and activation of FAK and ERK1/2 $\rightarrow$ disruption of intracellular contractile signaling (potentially involving calcium regulation or myosin light chain phosphorylation) $\rightarrow$ phase shift of detrusor contraction relative to the sphincter $\rightarrow$ decreased CCI $\rightarrow$ DSD phenotype [30].

Mirabegron is a β 3-adrenergic receptor agonist clinically used to treat overactive bladder [24]. The present study found that mirabegron restored the CCI under pathological conditions in a concentration-dependent manner (from 0.26 to 0.75 at 10 μ M), an effect comparable to that of a CTGF neutralizing antibody. Mechanistically, β 3-AR activation increases intracellular cAMP, which in turn regulates smooth muscle relaxation [31]. The advantage of combination therapy lies in simultaneously targeting

neural regulation ($\beta 3$ -AR) and local paracrine signaling (CTGF), which may be particularly beneficial for patients with potential downregulation of $\beta 3$ -AR expression after neural injury, where the CTGF pathway can serve as a synergistic target. Compared with existing treatments (α -blockers, anticholinergic drugs, and botulinum toxin), targeting the CTGF/integrin $\alpha v\beta 3$ pathway has not yet been attempted in DSD [24], and this study provides a theoretical basis for developing novel biologics or small-molecule inhibitors [32].

It should be noted that $\beta 3$ -adrenergic receptor ($\beta 3$ -AR) expression was not directly measured in the printed detrusor cells or dual-tissue constructs in this study. Therefore, the possibility that the observed restoration of CCI by mirabegron may involve off-target effects cannot be definitively excluded. However, substantial evidence from the literature supports the presence of functional $\beta 3$ -AR in bladder detrusor smooth muscle. At the mRNA level, $\beta 3$ -AR is the predominant β -adrenergic receptor subtype expressed in the human detrusor, accounting for approximately 97% of total β -AR mRNA [33]. At the protein level, $\beta 3$ -AR expression has been confirmed in human detrusor smooth muscle by immunohistochemistry and Western blotting. In cultured human and rat detrusor smooth muscle cells, $\beta 3$ -AR expression is maintained and functional, as evidenced by mirabegron-induced cAMP accumulation and cell relaxation. Furthermore, mirabegron is clinically approved for overactive bladder on the basis of its selective $\beta 3$ -AR agonism in the detrusor, providing indirect but strong evidence for $\beta 3$ -AR expression in the bladder wall [34]. Nevertheless, future studies should verify $\beta 3$ -AR expression in the printed detrusor constructs by quantitative PCR and immunofluorescence staining, and confirm target specificity using $\beta 3$ -AR-selective antagonists (e.g., L-748,337) or siRNA-mediated $\beta 3$ -AR knockdown in the bioprinted model. Importantly, as the present study did not directly measure $\beta 3$ -AR expression in the printed detrusor cells, future studies employing selective $\beta 3$ -AR antagonists (e.g., L-748,337) or $\beta 3$ -AR knockdown (siRNA) in the bioprinted model are warranted to definitively confirm target specificity.

Study limitations and future directions

Limitations: Fibroblasts were used for the urethral sphincter components instead of the striated muscle cells that actively contract physiologically. This allowed evaluation of the output of the paracrine signal, but it did not completely mimic the active contractile function of the sphincter. This model does not incorporate neural network and vascular network, unlike actual *in vivo* microenvironment^[35]. The direct effect of CTGF on the oscillation of calcium and/or myosin light chain phosphorylation in the detrusor cells through the FAK/ERK pathway is not directly determined. Translation evidence in the clinical setting is still scarce and the feasibility of CTGF for use as biomarker should be validated on a large scale. Additionally, the molecular mechanism by which ERK1/2 phosphorylation leads to detrusor smooth muscle cell asynchronous contraction is not yet clear, although this study demonstrates that CTGF activates the FAK/ERK pathway. Several plausible hypotheses merit consideration: (i) ERK may directly or indirectly phosphorylate myosin light chain kinase (MLCK) and/or the regulatory myosin light chain (MLC20), altering the force-generating capacity and rhythmicity of individual smooth muscle cells^[36]; (ii) ERK activation may modulate intracellular calcium homeostasis by regulating the activity of L-type voltage-gated calcium channels (Cav1.2), store-operated calcium entry (SOCE), or sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA), thereby desynchronizing the calcium oscillations that drive rhythmic contraction^[37]; and (iii) ERK signaling may downregulate the expression or phosphorylation of gap junction proteins, particularly Connexin 43 (Cx43), which is the predominant gap junction subunit in detrusor smooth muscle and is essential for electrical coupling and synchronous contraction of the bladder wall. Of these, the gap junction hypothesis is particularly attractive, as Cx43 downregulation has been documented in bladder dysfunction models and ERK-dependent Cx43 phosphorylation at serine residues (e.g., Ser279/282) is known to reduce gap junction communication^[38]. These hypotheses should be tested in future studies using calcium imaging, patch-clamp electrophysiology, and Cx43-specific interventions (e.g., Gap27 peptide, Cx43 overexpression) in the bioprinted model^[39,40].

Future directions: Construct a three-component model integrating neurons, muscle, and

fibroblasts by incorporating motor neurons and sphincter striated muscle cells ^[25] to investigate the crosstalk between neural and paracrine signals. Achieve vascularization and immune cell coculture to simulate the impact of inflammatory infiltration on the paracrine network after SCI ^[41]. Record electrophysiological changes in detrusor cells upon CTGF stimulation using calcium imaging or patch-clamp techniques. Leverage the high-throughput capacity of this model to screen small-molecule inhibitors of the CTGF/ α v β 3 pathway and determine the optimal ratio for combination with mirabegron. Conduct multicenter clinical studies to measure CTGF levels in urine or tissues of DSD patients at different stages and establish a non-invasive diagnostic model.

5. Conclusion

In this study, we developed a spatially defined bioprinting strategy and established the first in vitro model for quantitative assessment of detrusor-sphincter contraction coordination. Using this platform, we revealed a novel paracrine-based mechanism of DSD: after spinal cord injury, the local pathological microenvironment induces aberrant CTGF secretion from sphincter fibroblasts ^[42], which activates the integrin α v β 3-FAK/ERK pathway and leads to detrusor dysregulation. We also validated the therapeutic potential of mirabegron combined with a CTGF neutralizing antibody. These findings offer new insights into DSD pathophysiology and provide a theoretical basis for therapies targeting inter-tissue communication.

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Conflict of interest

The authors declare there are no competing interests.

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Ethics approval and consent to participate

This study involved isolation of primary fibroblasts from rat urethral sphincter tissue and the use of commercial rat primary bladder smooth muscle cells. All animal procedures were approved by the Animal Ethics Committee of Shanxi Medical University (approval number: 2026(675)). The study complied with the Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data

The data used in this study (including raw time-lapse contraction images, Western blot bands, qPCR data, etc.) are available from the corresponding authors upon reasonable request.

References

[1] Pereira JA, Debugne T. Evaluation Methods of Detrusor Sphincter Dyssynergia in Spinal Cord Injury Patients: A Literature Review. *Uro.* 2022;2(2):122-133. doi:10.3390/uro2020015

[2] Furrer MA, Kessler TM, Panicker JN. Detrusor Sphincter Dyssynergia. *Urol Clin North Am.* 2024;51(2):221-232. doi:10.1016/j.ucl.2024.01.001

[3] Feloney MP, Leslie SW. Bladder Sphincter Dyssynergia. [Updated 2023 Nov 12]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from:

<https://www.ncbi.nlm.nih.gov/books/NBK562166/>

- [4] Ruan J, et al.. Decoding the ceRNA regulatory landscape: a comprehensive transcriptomic analysis of ncRNA-driven bladder fibrosis following spinal cord injury. *Transl Androl Urol*. 2025;14(10):3003-3022. doi:10.21037/tau-2025-376
- [5] Wyndaele M, et al.. Beyond the urothelium: Interplay between autonomic nervous system and bladder inflammation in urinary tract infection, bladder pain syndrome with interstitial cystitis and neurogenic lower urinary tract dysfunction in spinal cord injury—ICI-RS 2023. *Neurourol Urodyn*. 2024;43(6):1283-1292. doi:10.1002/nau.25310
- [6] Matsuta Y, Yoshioka K, Tainaka H, et al.. Effect of amniotic fluid stem cell transplantation on the recovery of bladder dysfunction in spinal cord-injured rats. *Sci Rep*. 2020;10(1):10030. doi:10.1038/s41598-020-67163-7
- [7] HUANG M.S., CHRISTAKOPOULOS F., ROTH J.G. & HEILSHORN S.C. (2025). Organoid bioprinting: from cells to functional tissues. *Nature Reviews Bioengineering*. 3, 126-142. doi:10.1038/s44222-024-00268-0
- [8] CAO S., MA H. & WU C. (2026). Biomaterials-modulated multicellular crosstalk in 3D bioprinted constructs. *Advanced Interventional Materials*. doi:10.1016/j.advim.2025.100008
- [9] Du L, Lu Y, Yang H. Three-dimensional (3D) bioprinted co-culture models: a new paradigm for reproducing the tumor microenvironment and precision therapy. *Hepatobiliary Surg Nutr*. 2025;14(3):482-485. doi:10.21037/hbsn-2025-297
- [10] Hu M, Dong L, Gao Y, et al. Prospects for 3D bioprinting research and transdisciplinary application to preclinical animal models. *Lab Anim Comp Med*. 2025;45(3):318-330. [In Chinese] doi:10.12300/j.issn.1674-5817.2024.193
- [11] Jeon S, Heo J-H, Kim MK, Jeong W&KangH-W. High-Precision 3D Bio-Dot Printing to Improve Paracrine Interaction between Multiple Types of Cell Spheroids. *Adv Funct Mater*. 2021;31(10):2005324. doi:10.1002/adfm.202005324
- [12] Yong U, Park J, Jang J. Bioprinted Excitable Tissues with Multistimulation Systems for Promoting Function and Maturation. *Advanced NanoBiomed Research*. 2025;6(3). doi:10.1002/anbr.202500107
- [13] REA M, DI LISAL, PAGNOTTA G, GALLO N, SALVATORE L, D'AMICO F, et al.. Establishing a Bioink Assessment Protocol: GelMA and Collagen in the Bioprinting of a Potential In Vitro Intestinal Model. *ACS Biomater Sci Eng*. 2025;11(4):2456-2467. doi:10.1021/acsbiomaterials.5c00034
- [14] JEONG W., HAN J., CHOI J. & KANG H.-W. (2024). Embedded Bioprinting of

Breast Cancer-Adipose Composite Tissue Model for Patient-Specific Paracrine Interaction Analysis. *Advanced Healthcare Materials*, e2401887. doi:10.1002/adhm.202401887

[15]BEN-GIDA H., GURKA R. & LIBERZON A. (2020). OpenPIV-MATLAB — An open-source software for particle image velocimetry; test case: Birds' aerodynamics. *SoftwareX*, 12, 100585. doi:10.1016/j.softx.2020.100585

[16]DICKMAN CTD, RUSSO V, THAIN K, PAN S, BEYER ST, WALUS K, GETSIOS S, MOHAMED T&WADSWORTH SJ. Functional characterization of 3D contractile smooth muscle tissues generated using a unique microfluidic 3D bioprinting technology. *FASEB J*. 2020;34(1):1652-1664. doi:10.1096/fj.201901063RR

[17]LEE H.L., KIM B., YUN J., BAE J., PARK S., JEON J., JANG H.R., LEE J. & LEE S. (2024). PIV-MyoMonitor: an accessible particle image velocimetry-based software tool for advanced contractility assessment of cardiac organoids. *Frontiers in Bioengineering and Biotechnology*, 12, 1367141. doi:10.3389/fbioe.2024.1367141

[18]SZABÓ A, PASQUARIELLO R, COSTA PF, et al.. Light-Based 3D Printing of Gelatin-Based Biomaterial Inks to Create a Physiologically Relevant In Vitro Fish Intestinal Model. *Macromol Biosci*. 2023;23(10):e2300016. doi:10.1002/mabi.202300016

[19]GUO DY, CHEN ZH, FU YF, LI YY, CHEN MN, WU JJ, YUAN ZD, YE JX, LI X&YUANFL. Cilengitide inhibits osteoclast adhesion through blocking the $\alpha\text{v}\beta 3$ -mediated FAK/Src signaling pathway. *Heliyon*. 2023;9(7):e17841. doi:10.1016/j.heliyon.2023.e17841

[20]Budharaju H, Singh RK, Kim HW. (2025). Bioprinting for drug screening: A path toward reducing animal testing or redefining preclinical research? *Bioactive Materials*, 51, 993-1017. doi:10.1016/j.bioactmat.2025.07.006

[21]Metcalf PD, Wang J, Jiao h, et.al.. (2010). Bladder outlet obstruction: progression from inflammation to fibrosis. *BJU Int*, 2010 Dec;106(11):1686-94. . DOI:10.1111/j.1464-410X.2010.09445.x

[22]Lee SY, et al.. Endothelial cell-derived connective tissue growth factor stimulates fibroblast differentiation into myofibroblasts through integrin $\alpha\text{V}\beta 3$. *Exp Ther Med* . 2022 Nov 24;25(1):30 DOI: 10.3892/etm.2022.11730

[23]TAN TW, LAI CH, HUANG CY, YANG WH, CHEN HT, HSU HC, FONG YC&TANGCH. CTGF enhances migration and MMP-13 up-regulation via $\alpha\text{v}\beta 3$ integrin, FAK, ERK, and NF- κB -dependent pathway in human chondrosarcoma cells. *J Cell Biochem*. 2009;107(2):345-356. doi:10.1002/jcb.22132

- [24]Tousignant A, Blais M-A, Tu L-M, Morin M&IsmailS. A Scoping Review of the Oral Treatment Options for the Management of Detrusor Sphincter Dyssynergia. *Neurourol Urodyn*. 2025;44(4):743-753. doi:10.1002/nau.25642
- [25]YILMAZ E.G., HACIOSMANOĞLU N., INCI F. & ASHAMMAKHI N. (2025). Modeling the synapse and neuromuscular junction using organ-on-a-chip technology. In *Handbook of Neural Engineering*, 625-643. Elsevier. DOI: 10.1016/B978-0-323-95730-4.00008-1
- [26]ALTUNTAS CZ, DANESHGARI F, IZGI K, BICER F, OZER A, SAKALAR C, GRIMBERG KO, SAYIN I, TUOHY VK. Connective tissue and its growth factor CTGF distinguish the morphometric and molecular remodeling of the bladder in a model of neurogenic bladder. *Am J Physiol Renal Physiol*. 2012 Nov 1;303(9):F1363-9. doi: 10.1152/ajprenal.00273.2012.
- [27]Yue K., Trujillo-de Santiago G., Alvarez M.M., Tamayol A., Annabi N., Khademhosseini A. (2015). Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials*, 73, 254–271. doi:10.1016/j.biomaterials.2015.08.045
- [28]Leask A, Abraham DJ. All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci*. 2006;119(23):4803–4810. doi:10.1242/jcs.03270
- [29]Mori T, Kawara S, Shinozaki M, Hayashi N, Kakinuma T, Igarashi A, Takigawa M, Nakanishi T, Takehara K. Role and interaction of connective tissue growth factor with transforming growth factor- β in persistent fibrosis: a mouse fibrosis model. *J Cell Physiol*. 1999;181(1):153–159. doi: 10.1002/(SICI)1097-4652(199910)181:1<153::AID-JCP16>3.0.CO;2-K
- [30]Kim HR, Appel S, Vetterkind S, Gangopadhyay SS, Morgan KG. Smooth muscle signalling pathways in health and disease. *J Cell Mol Med*. 2008;12(6A):2165–2180. doi:10.1111/j.1582-4934.2008.00552.x
- [31]Maki T, Kajioka S, Itsumi M, Kareman E, Lee K, Shiota M&EtoM. Mirabegron induces relaxant effects via cAMP signaling-dependent and -independent pathways in detrusor smooth muscle. *Low Urin Tract Symptoms*. 2019;11(2):O209-O217. doi:10.1111/luts.12247
- [32]Yamaguchi O. β 3-adrenoceptors in human detrusor muscle. *Urology*. 2002;59(5 Suppl 1):25–29. doi:10.1016/S0090-4295(01)01635-1
- [33]Lee JShinH. Composite Spheroid-Laden Bilayer Hydrogel for Engineering Three-Dimensional Osteochondral Tissue. *Tissue Eng Part A*. 2024;30(5-6):225-243.

doi:10.1089/ten.TEA.2023.0299

[34]Berridge MJ. Smooth muscle cell calcium activation mechanisms. *J Physiol.* 2008;586(21):5047–5061. doi:10.1113/jphysiol.2008.160440

[35]Rayego-Mateos SRuiz-OrtegaM. Interplay between extracellular matrix components and cellular and molecular mechanisms in kidney fibrosis. *Clin Sci (Lond).* 2021;135(16):1999-2029. doi:10.1042/CS20201016

[36]Martin-Aragon Baudel M Navedo MF. (2020). Purinergic Signaling During Hyperglycemia in Vascular Smooth Muscle Cells. *Front Endocrinol (Lausanne)*, 11, 329. doi:10.3389/fendo.2020.00329

[37]Nakamura AKohamaK. Role of non-kinase activity of myosin light-chain kinase in regulating smooth muscle contraction, a review dedicated to Dr. Setsuro Ebashi. *Biochem Biophys Res Commun.* 2008;369(1):135-43. doi:10.1016/j.bbrc.2007.11.096

[38]Neuhaus JDorschnerW. Smooth muscle cells from human urinary bladder express connexin 43 in vivo and in vitro. *World J Urol.* 2002;20(4):250-4. doi:10.1007/s00345-002-0289-9

[39]D'hondt CBultynckG. Regulation of connexin- and pannexin-based channels by post-translational modifications. *Biol Cell.* 2013;105(9):373-98. doi:10.1111/boc.201200096

[40]Nomiya MYamaguchiO. A quantitative analysis of mRNA expression of alpha 1 and beta-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. *J Urol.* 2003;170(2 Pt 1):649-53. doi:10.1097/01.ju.0000067621.62736.7c

[41]Zhang J, Ren F, Bu F, Yao Y, Li M. 3D bioprinting-driven strategies for tissue regeneration and controlled immune modulation. *Int J Bioprint.* 2026;12(1):173-198. doi:10.36922/IJB025460474

[42]Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. *Arch Phys Med Rehabil.* 2007;88(11):1384–1393. DOI: 10.1016/j.apmr.2007.08.004