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BRIEF COMMUNICATION

Improved symptoms and signs of refractory interstitial cystitis in women after intravesical Nanofat plus platelet-rich plasma grafting: A pilot study

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Abstract: Interstitial cystitis/bladder pain syndrome (IC/BPS) is characterized by bladder pain accompanied by irritative urinary symptoms, and typical cystoscopic and histological features. In this pilot study, we assessed the impact of lesion-targeted bladder injection therapy using a biocellular regenerative medicine on patients with refractory IC/BPS. The medicine, which was an autologous emulsified fat (Nanofat) and platelet-rich plasma (PRP) combination, was prepared intraoperatively. Six patients (aged 40-54 years), who completed a standard protocol of four consecutive treatments at 3-month intervals, were followed up at 6 months postoperatively. All patients (100%) reported marked (+3; +3 ~ −3) improvement of their overall bladder conditions. Mean bladder pain (from 8.2 to 1.7; range: 0 ~ 10), IC-related symptoms (from 18.5 to 5.7; range: 0 ~ 20), and bother (from 14.8 to 3.8; range: 0 ~ 16) improved significantly ($p < 0.01$). The normalization of bladder mucosal morphology with treatments was remarkable under cystoscopic examination, and no significant adverse events were found. The cultured mesenchymal stem cells from Nanofat samples of the six patients were verified in vitro. Our preliminary results suggest novel intravesical therapy with autologous Nanofat plus PRP grafting is safe and effective for refractory IC/BPS. Surgical efficacy might be attributed to an in vivo tissue engineering process.

Keywords: Interstitial cystitis/bladder pain syndrome (IC/BPS); Intravesical therapy; Mesenchymal stem cells; Nanofat; Platelet-rich plasma; Stromal vascular fraction

1. INTRODUCTION

Painful bladder sensation accompanied by irritative urinary symptoms is the main complaints of patients with interstitial cystitis/bladder pain syndrome (IC/BPS). Further typical cystoscopic and histological features are needed for the diagnosis and subclassification of IC/BPS.[1] The treatment of IC/BPS is problematic because the causes are not fully understood.[2]

Currently, the most accepted postulate for the pathogenesis of IC/BPS is a dysfunctional urothelium from bladder insults and the penetration of urine toxins and pathogens leads to inflammation, immunogenic responses, and neural sensitization in these patients.[3] Intravesical glycosaminoglycan replenishment therapy had been used to restore the barrier function of urothelium and provide symptomatic relief

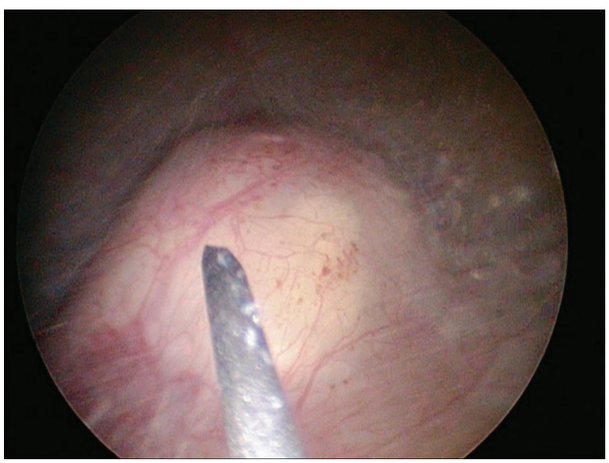

Fig. 1 Submucosal retention of the injected biocellular regenerative medicine containing Nanofat and platelet-rich fibrin after the infusion of a 10% calcium chloride solution at the bladder recipient site

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Table 1

Patient characteristics

Patient	Age (y)	BMI (kg/m^2)	Body fat %	Duration (mo)	[a]ESSIC typing	[b]Treatment
1	47	21.0	28.1	26	2	BoNT-A X 1
2	43	21.6	30.9	35	2	BoNT-A X 3
3	54	23.6	34.5	14	2	HA only
4	47	24.7	35.3	46	2	BoNT-A X 4
5	47	21.4	35.8	15	3	Electrofulguration X 3
6	40	27.7	38.7	12	2	BoNT-A X 2
Average	46.3 ± 4.7	23.3 ± 2.6	33.9 ± 3.8	24.7 ± 13.6		

BoNT-A = Botulinum toxin A; ESSIC = European Society for the Study of Interstitial Cystitis; HA = hyaluronic acid; IC/BPS = interstitial cystitis/bladder pain syndrome.

[a]One (16.7%) patient (No. 5) was diagnosed with ESSIC type 3 (ulcerative) IC/BPS.

[b]Prior treatments included monthly HA instillation in six (100%), repeated (1-4) sessions of BoNT-A bladder injection every 6 months in four (66.7%), and repeated (3) sessions of electrofulgurations of Hunner's lesions in one (16.7%) patient, respectively.

in these patients. However, the therapy faces major limitations of a high proportion (~30%-40%) of nonresponders.[4–6] Clinical trials of repeated intravesical Botulinum toxin A injections in patients with refractory IC/BPS have shown positive therapeutic effects because of sensory inhibitory and anti-inflammatory effects in addition to motor effects. However, postoperative urinary tract infections and voiding difficulty raised concerns.[4,7,8]

Recently, the potential applications of regenerative medicine, such as platelet-rich plasma (PRP) and stem cells, have been shown to be beneficial to treatment of IC/BPS in some preclinical and few clinical studies.[9–12] PRP, which contains several growth factors and signal proteins released by actively degranulated platelets, acts on available cells to begin the tissue repair and regeneration processes.[9,10] Nanofat, which is a stromal vascular fraction (SVF) obtained from mechanical emulsification and filtration of adipose tissue and contains a heterogeneous population of stem/progenitor cells and extracellular matrix, is a therapeutic paradigm in regenerative medicine.[13–15] In plastic surgery, Nanofat is an injectable viscous extract that primarily induces tissue remodeling after grafting. In contrast, Macro- and Microfat grafts, which contain mainly small lobules of adipose tissue and viable adipocytes, are used as a filling material for soft-tissue defects.[16] Combined Nanofat and PRP grafting, which was thought to work better than either alone because of a biocellular synergistic effect, has been used in skin rejuvenation, treatment of scars and infected ulcers, etc.[17–21]

We hypothesized that a bladder injection therapy using Nanofat and PRP combination might contribute to an in vivo tissue engineering process at the recipient sites and result in symptomatic and morphological improvement in patients with IC/BPS. In this pilot study, we aimed to evaluate the impact of this novel intravesical therapy using biocellular regenerative medicine on our patients with refractory IC/BPS.

2. METHODS

Between February and September 2019, a total of seven women with refractory IC/BPS were enrolled consecutively for this study. Informed consent was obtained. The Institutional Review Board and Ethics Committee approved this clinical trial (CSMUH No: CS1-20188).

One patient (14.3%) was excluded due to inadequate fat harvest from liposuction. Six patients, who completed a standard protocol of four consecutive bladder injection therapies at 3-month intervals, were followed up. Pain Visual Analog Scale (pain-VAS), Interstitial Cystitis Symptom and Problem Index (ICSI and ICPI), and a scaled Global Response Assessment (GRA) were used for assessing outcomes. Cystoscopic hydrodistention was performed before injections to localize various bladder mucosal lesions for a lesion-targeted treatment and to evaluate morphological responses from prior treatments.

The preparation of Nanofat grafts was conducted according to the methods described by Tonnard et al[13] with some modifications using industry-manufactured specific devices (Tulip Medical Products; San Diego, CA, USA). For the preparation of PRP, a commercial device (Tropocells PRP systems; Estar Medical, Holon, Israel) was used. Approximately, 12-mL Nanofat and 8-mL PRP were obtained from 60-mL lipoaspirate and 20-mL whole blood, respectively. The mixture (~20 mL) was used for a lesion-targeted bladder injection at 10 sites (2 mL at each site). After grafting, PRP was converted into fibrin matrix by the infusion of a 10% calcium chloride solution in a 1:10 ratio to the recipient sites to facilitate the submucosal retention of the grafts[22] (Fig. 1).

Table 2

Treatment outcomes

	GRA	ICSI		ICPI		Pain-VAS		Cystoscopic capacity (mL)	
Patient	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
1	+3	18	3	16	1	5	1	980	1000
2	+3	19	6	16	4	6	2	950	750
3	+3	16	0	14	0	8	0	870	970
4	+3	20	11	15	7	10	3	870	980
5	+3	19	5	16	4	10	1	530	600
6	+3	19	9	12	7	10	3	625	750
Average	+3	18.5 ± 1.4	5.7 ± 4.0	14.8 ± 1.6	3.8 ± 2.9	8.2 ± 2.2	1.7 ± 1.2	804.2 ± 183.4	841.7 ± 164.9
p		<0.001		<0.001		<0.001		0.49	

GRA = global response assessment (+3 ~ −3); ICPI = interstitial cystitis problem index (+16 ~ 0); ICSI = interstitial cystitis symptom index (20~0); Pain-VAS = pain visual analog scale (+10 ~ 0).

3. RESULTS

Patient characteristics are shown in Table 1. Our patients were characterized by having unsatisfactory results to multiple lines of standard treatments at a mean duration of 24.7 ± 13.6 months (range, 12-46 months).

Six months postoperatively, all patients (100%) reported marked (+3; +3 ~ −3) improvement in their overall bladder conditions on the GRA. Statistically significant ($p < 0.01$) improvement was also found from the changes of various pain-VAS, ICSI, and ICPI scores before and after treatments (Table 2). There were no significant adverse events except for subcutaneous ecchymosis from liposuction.

Progressive remission of various bladder mucosal lesions during treatments and at 6 months postoperatively was noted in all six patients (100%) (Figs. 2 and 3). The cell yield of Nanofat samples from our six patients is shown in Table 3. Mesenchymal stem cells (MSCs) cultured from Nanofat samples of the six patients were characterized in vitro via standard operating procedures according to an official recommendation[23,24] (Fig. 4).

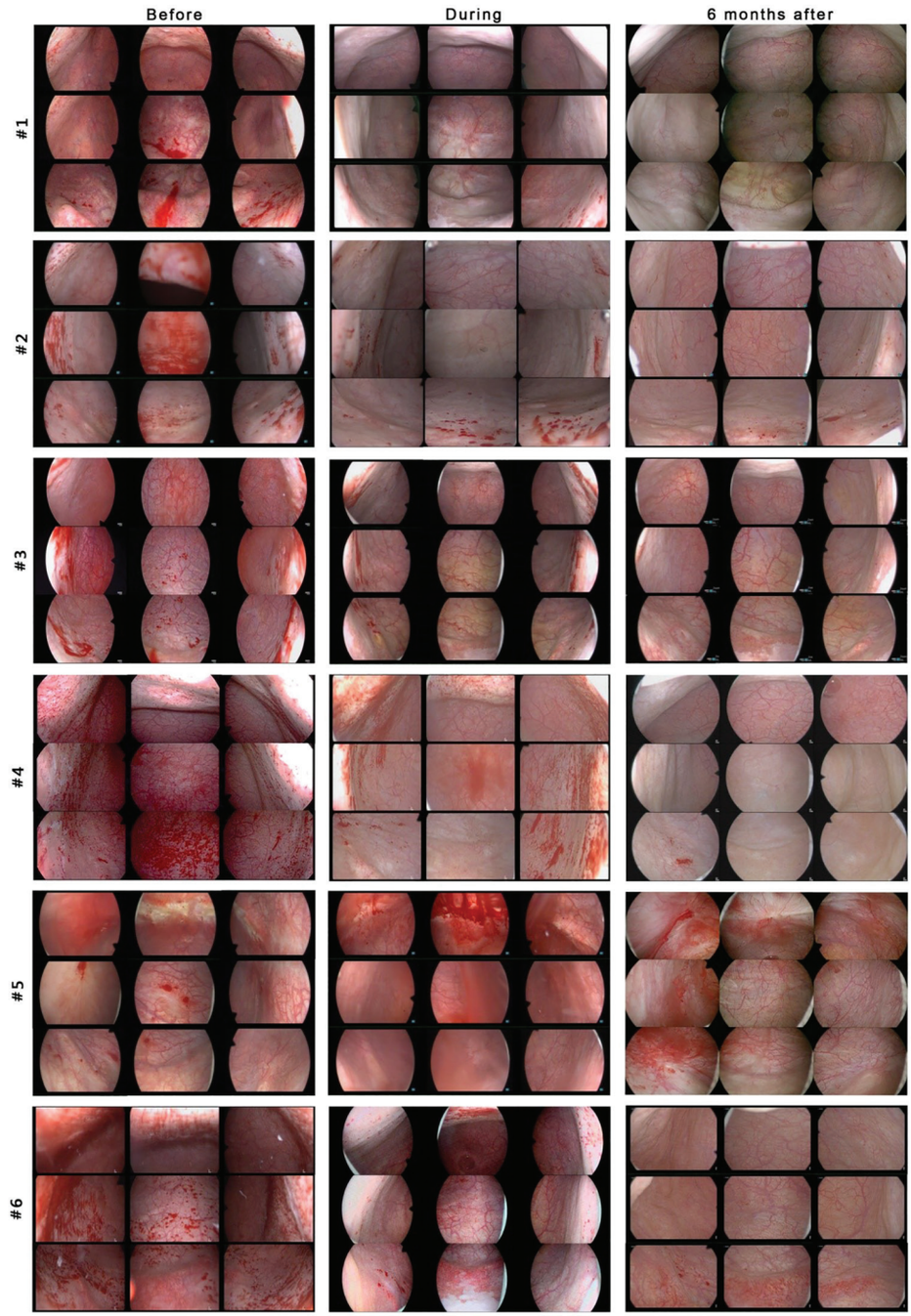

Fig. 2 Bladder morphological outcomes during treatments and at 6 mo postoperatively. Progressive normalization (from left to right) of bladder mucosal morphology was noted in all six (100%) patients

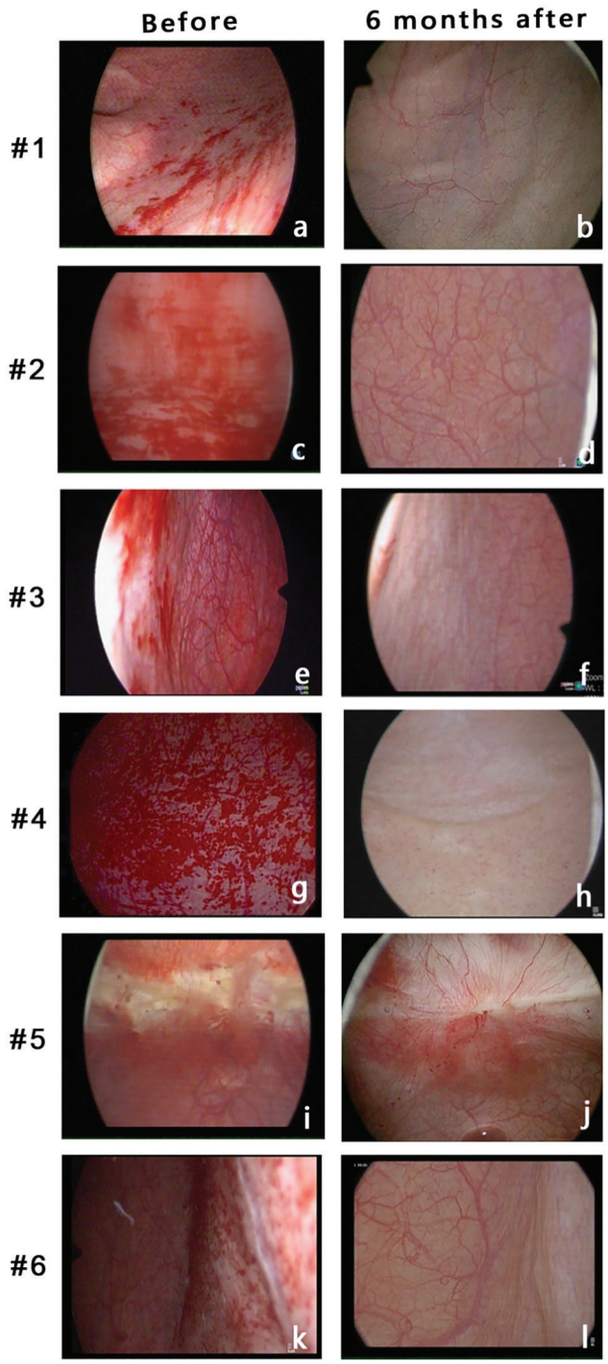

Fig. 3 The remission of various bladder mucosal lesions, ie, glomerulations (A-H), mucosal disruption (I, J) and fissures (K, L), after lesion-targeted bladder injection therapy with Nanofat plus platelet-rich plasma grafting

4. DISCUSSION

Until now, there have been few clinical trials using regenerative medicine for treating IC/BPS. Lander et al[12] assessed the impact of combined intravenous and local injection of autologous SVF stem cells on 109 patients with IC/BPS and found the treatment was safe and effective. However, there was no objective outcome measurement and no reporting whether repeated treatments were necessary. Jiang et al[10] performed repeated bladder injection therapy using autologous PRP followed by immediate cystoscopic hydrodistention at monthly intervals in 40 patients with refractory IC/BPS and found improved symptoms and altered urinary functional proteins after treatment. However, no morphological results were reported.

In this study, the normalization of bladder mucosal morphology with consecutive treatments indicated a tissue repair and regeneration therapeutic effect. This finding also supported our hypothesis that the novel intravesical therapy might contribute to an in vivo tissue engineering process since the grafts contained fundamental elements, ie, stem cells, growth factors, and extracellular matrix, required for the activity. In the future, further studies are necessary to determine whether the clinical observations can be correlated with histologic analysis before and after treatment.

Additionally, we performed laboratory analysis of Nanofat samples from our six patients. The estimated yield of SVF cells and MSCs per milliliter of Nanofat was 310 (±170) × 10^3 and 14 (±5) × 10^3, respectively. Our yield of SVF cells was higher than that reported by Gentile et al.[25] In their study, a yield of 20 ~ 200 (±3-15) × 10^3 SVF cells per milliliter of Nanofat obtained from four different methods was found. Besides, a positive correlation between the SVF cell yield and clinical outcomes was noted when using Nanofat grafts for treatment of scars.[25] Accordingly, the relatively high SVF cell yield of our Nanofat might, therefore, contribute to the promising results in this study.

The weakness of this study was the small sample size and a lack of control study. The strength of this study was the sequential follow-up of morphological responses from consecutive treatments.

In conclusion, our preliminary results suggested autologous Nanofat plus PRP grafting is safe and effective for IC/BPS. Morphological outcomes supported our hypothesis that surgical effectiveness might be attributed to an in vivo tissue engineering process.

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Table 3

Analysis of the cell yield of Nanofat samples from six patients with refractory IC/BPS

Patient	Mass of tested Nanofat (mL)	Yield [a]total nucleated cells /mL Nanofat (×10^5)	Yield [b]adherent cells/mL Nanofat (×10^5)	Adherent cells/total nucleated cells (%)
1	12.0	1.6	0.11	6.9%
2	1.0	1.0	0.06	6.0%
3	2.0	4.7	0.19	4.0%
4	2.0	3.4	0.14	4.1%
5	1.5	5.2	0.19	3.7%
6	2.0	2.9	0.12	4.1%
Average	3.42 ± 4.2	3.13 ± 1.7	0.135 ± 0.05	4.3 ± 1%

IC/BPS = interstitial cystitis/bladder pain syndrome; SVF = stromal vascular fraction.

[a]Total nucleated cells: the SVF cells.

[b]Adherent cells: the putative mesenchymal stem cells.

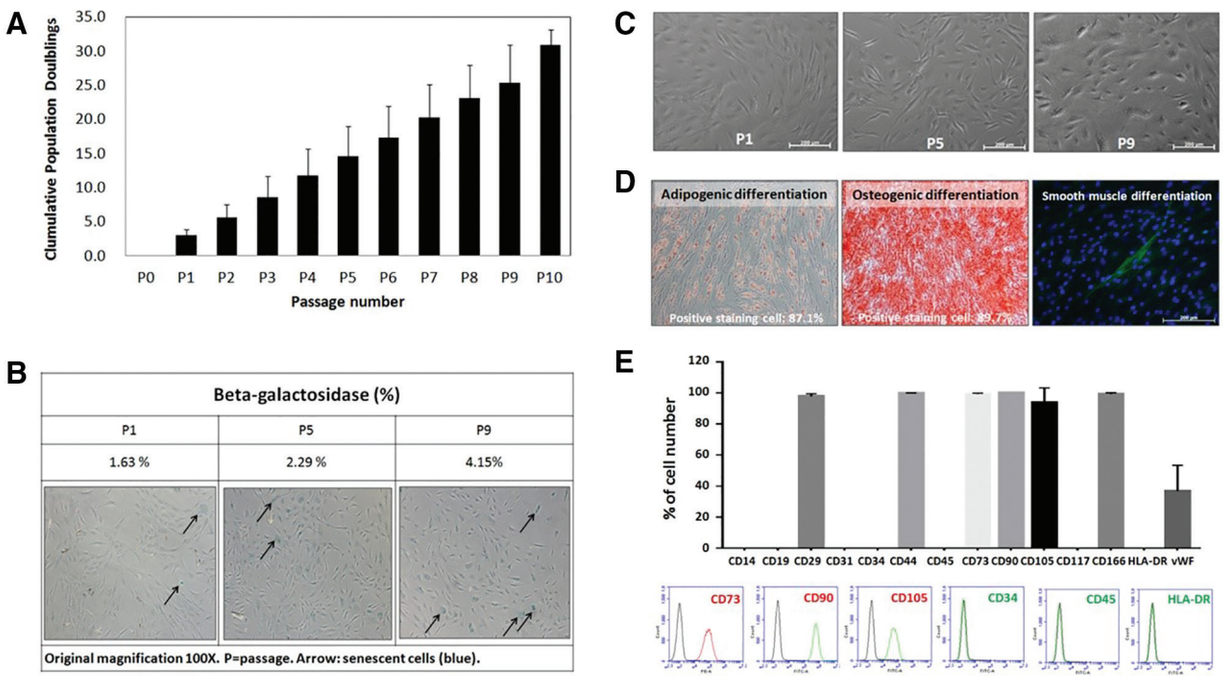

Fig. 4 In vitro characterization of cultured mesenchymal stem cells from Nanofat samples of six patients who underwent the novel bladder injection therapy. A, The growth kinetics by cumulative population doublings. B, Senescence assay by beta-galactosidase staining of cultured mesenchymal stem cells. P: passage. Arrow: senescent cells (blue). C, Microscopic cell morphology. Original magnification ×100. D, Trilineage differentiation potential by cytochemical staining with Oil Red-O (adipogenic), Alizarin Red S (osteogenic), and by immunofluorescent staining to α-smooth muscle actin (smooth muscle differentiation). Original magnification ×100. E, Detection of cell surface antigen by flow cytometry

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