



Gotoh M, Yamamoto T, Matsukawa Y, Funahashi Y

Department of Urology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Hypothesis / aims of study

We created a novel treatment strategy to regenerate the urethral sphincter function, using autologous adipose-derived regenerative cells (ADRCs) without the need of cell culture (Fig.1). We previously demonstrated the safety and favorable long-term efficacy of periurethral injection of autologous ADRCs for the treatment of male stress urinary incontinence (SUI) following prostatectomy [1]. Briefly, SUI improved progressively in eight patients (72.7%) during the 1-year follow up, as determined by a 59.8% decrease in the leakage volume in the 24-h pad test, decreased frequency and amount of incontinence, and improved quality of life. One patient obtained complete continence at 6 months after treatment. In the present study, we report the short-term outcome (9 to 12 months) of the first three female patients with SUI undergoing periurethral injection of autologous ADRCs.

[1] Gotoh M, Yamamoto T, et al: Regenerative treatment of male stress urinary incontinence by periurethral injection of autologous adipose-derived regenerative cells: 1-year outcomes in 11 patients. Int J Urol. 2014 Mar;21(3):294-300

Study design, materials and methods

Three female patients with SUI were included in this preliminary clinical trial. No patients had urgency incontinence. After liposuction of 250 mL of adipose tissue from the abdomen, we isolated ADRCs by using the Celution™ system. Subsequently, these ADRCs and a mixture of ADRCs and adipose tissue were transurethrally injected into the rhabdosphincter and submucosal space of the urethra, respectively. Unlike other cell therapies, this treatment is entirely autologous, does not require cell culture, and is performed as a single surgical procedure. In the protocol, the primary outcome is assessed at by a 24-hour pad test, at baseline, 2 weeks, 1 month, and every 3 months thereafter until 36 months after treatment. Secondary outcomes included a validated patient questionnaire (the International Consultation on Incontinence Questionnaire-Short Form; ICIQ-SF), urethral pressure profile, contrast-enhanced transvaginal ultrasonography, and magnetic resonance imaging (MRI). Here, we report the outcomes of treatment efficacy and safety at the 9-12 month follow-up in the 3 cases.

The present study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine, and also by the committee of Japanese Ministry of Health, Labor and Welfare according to the Guidelines on Clinical Research using Human Stem Cells. Written informed consent was obtained from the patients.

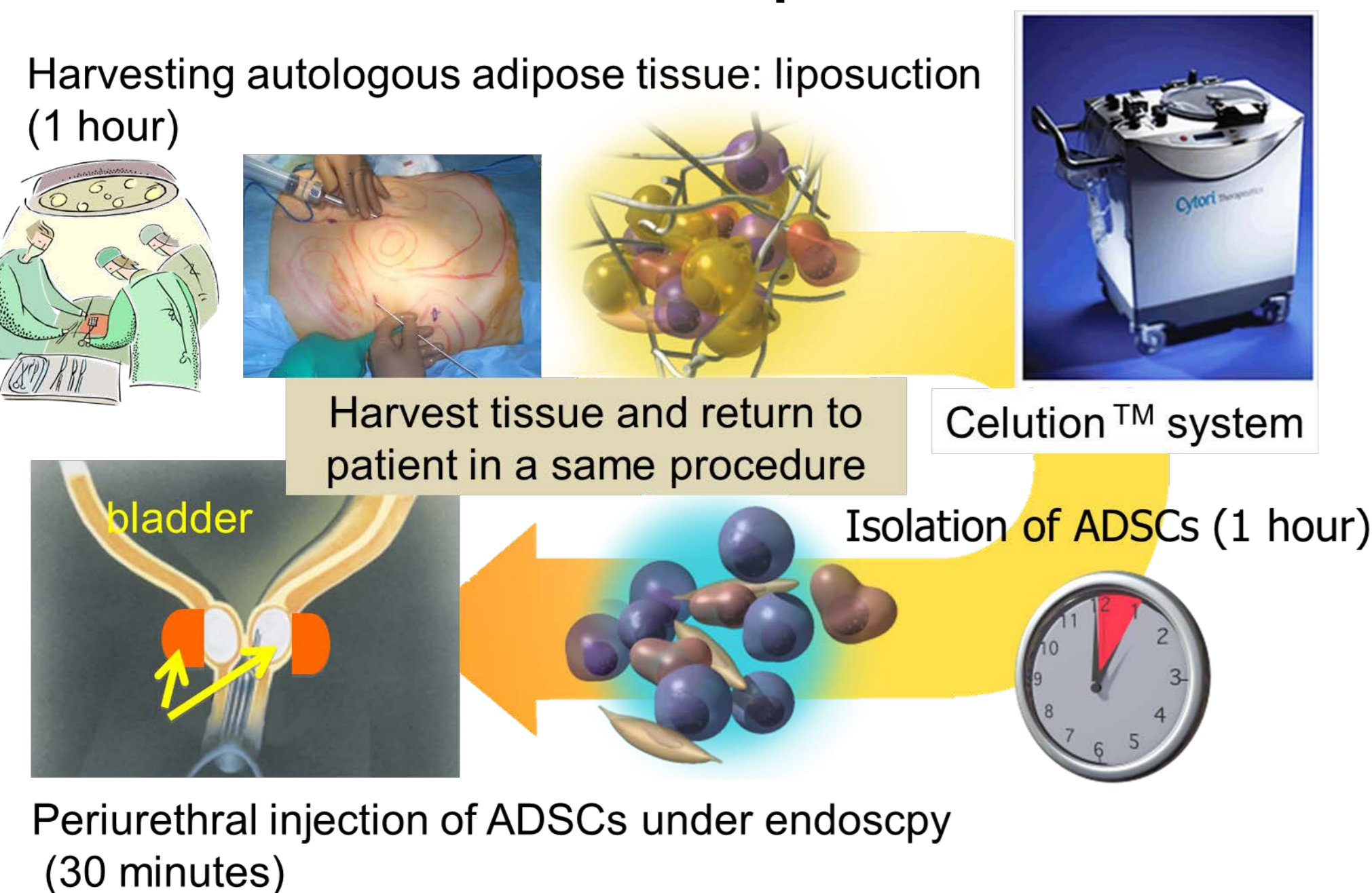
Patients

In the present study, 3 patients with uncomplicated genuine SUI were enrolled. Patients' ages were 40, 39, and 64 years, respectively. No patients had a history of previous surgery for SUI and co-existing disorders.

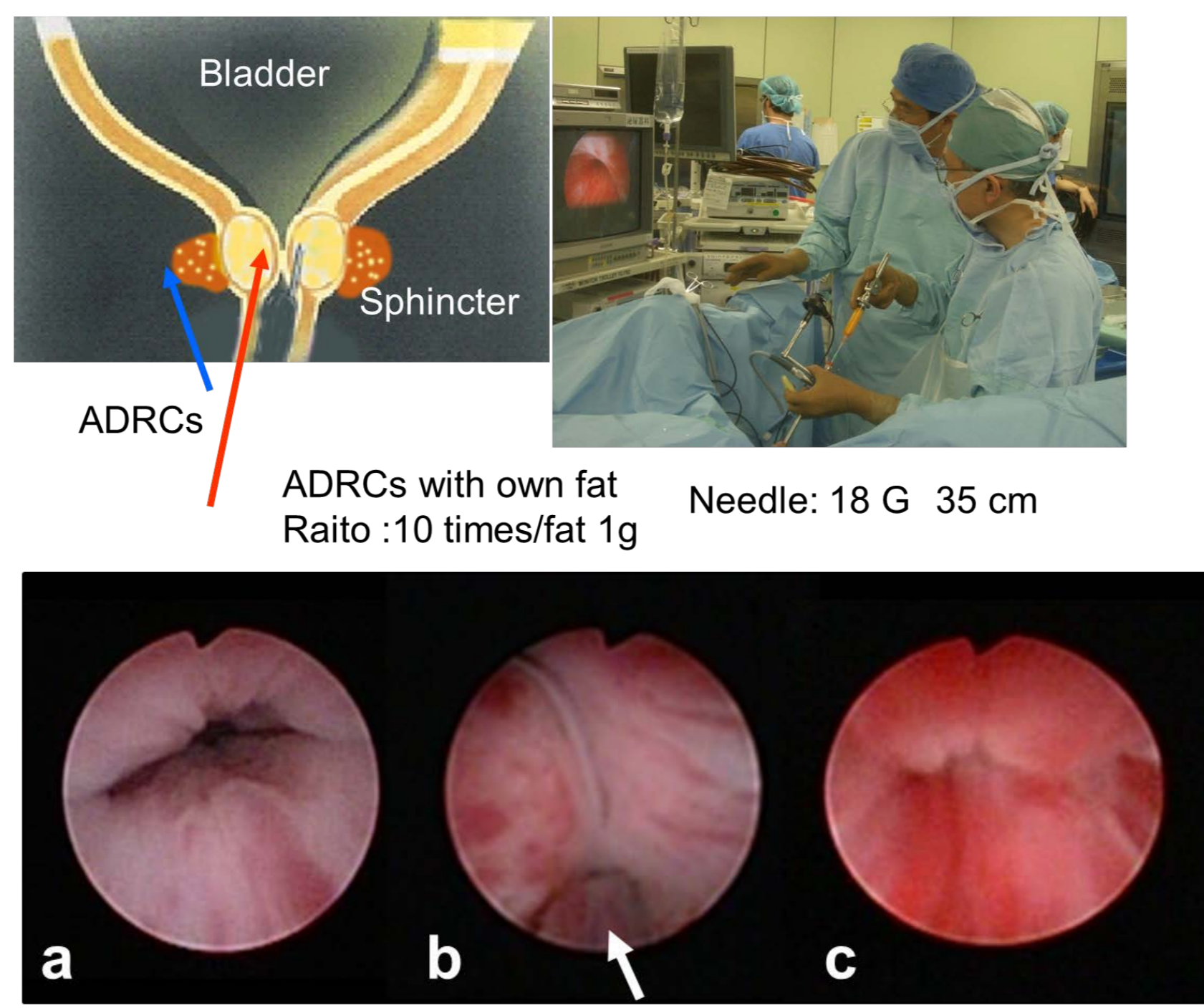
Harvesting adipose tissue and isolation of ADRCs

After liposuction of 250 ml of adipose tissue from the anterior abdominal wall, ADRCs were isolated from the harvested adipose tissue by using the Celution™ system. Briefly, adipose tissue was introduced into the Celution™ cell-processing device which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwarranted or deleterious cells, cell and matrix fragments. It required around 1 hour to process 250 mL of liposuction tissue. The final concentrated cell output collected using the Celution™ System was counted using a NucleoCounter (Chemometec, Denmark), which exclusively detected nucleated cells. By using the Celution™ system, we could finally obtain a 5 mL solution containing concentrated ADRCs.

A flow chart of the procedures



Endoscopic periurethral injection of ADRCs



The urethral lumen at the region of the external urethral sphincter remained open (a), as observed via endoscopy. ADRCs and ADRCs with own fat were injected into the external sphincter and submucosal spaces, respectively, through an injection needle (b). The urethral lumen completely closed after periurethral injection (c). Arrow: an injection needle

Outcome measures

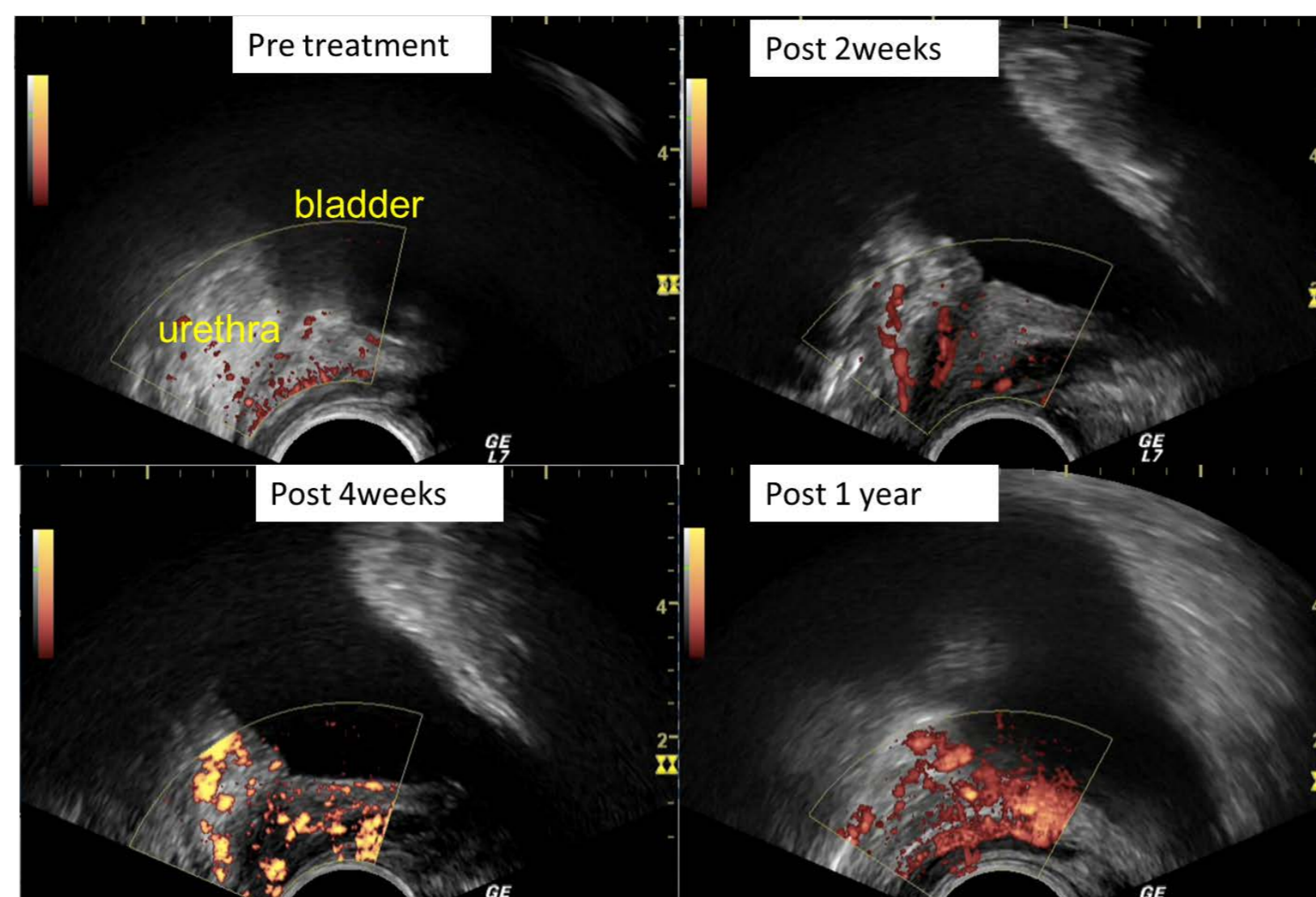
1. amount of incontinence: The 24-hour pad test was consecutively repeated for 4 days for each evaluation period. The total daily leakage amount was calculated as mean during 4 days at each period.
2. Subjective symptoms and QOL: evaluated using a validated disease-specific questionnaire—the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF)
3. The urethral sphincter function: objectively assessed by measuring the urethral pressure profile; maximum urethral closing pressure (MUCP) and functional profile length (FPL).
4. Blood flow to the area where ADRCs were injected: assessed by contrast-enhanced transvaginal ultrasonography by intravenously injecting perflubutane.
5. Morphological condition of the injected area: monitored by magnetic resonance imaging (MRI).
5. Adverse events
6. Uroflowmetry and measure of residual urine volume

Results

Outcomes on isolation and injection of ADRCs

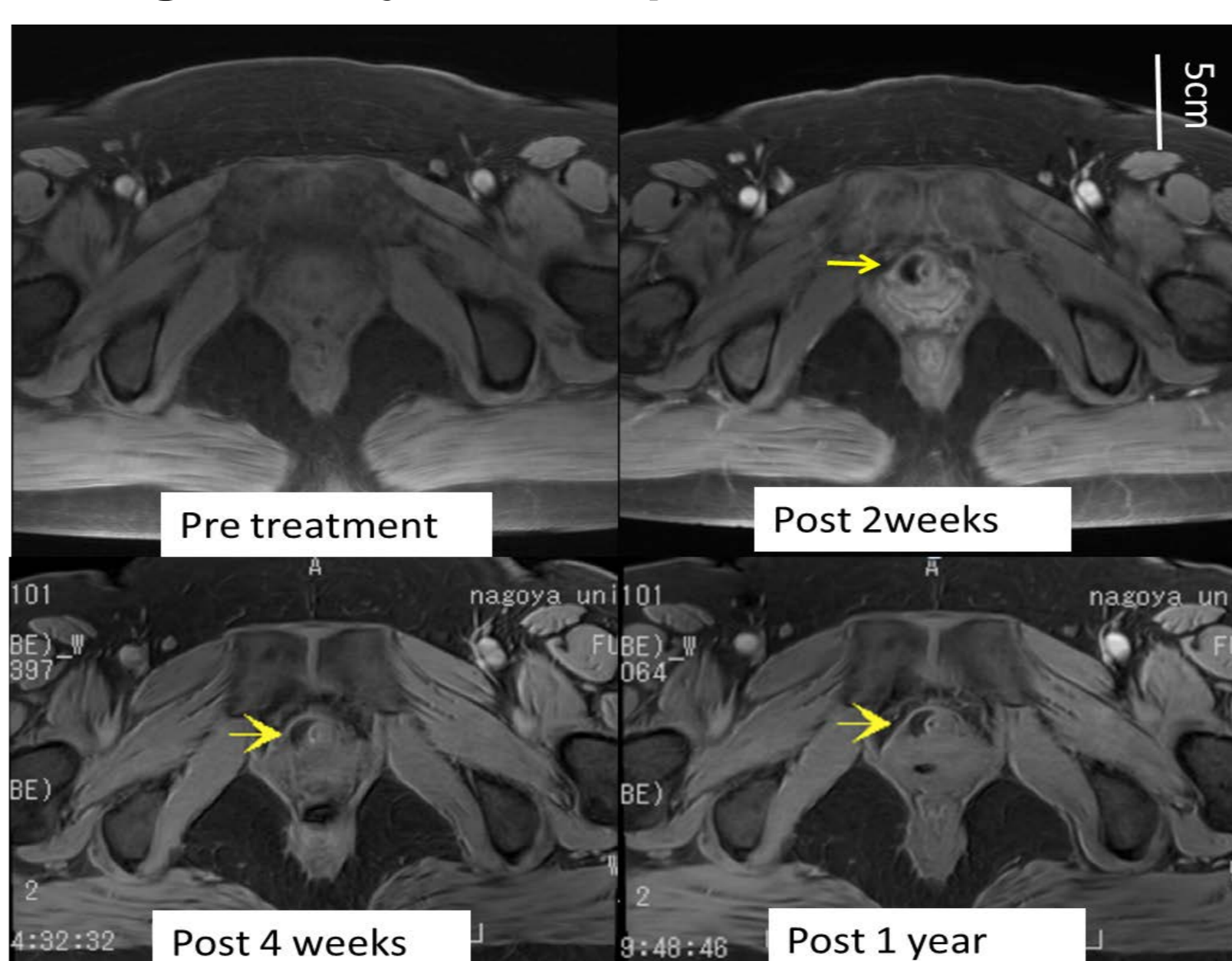
| Patients | Age | Time for ADRCs isolation (minutes) | Time for ADRCs injection (minutes) | No. of isolated cells | % of viable cells |
|----------|-----|------------------------------------|------------------------------------|-----------------------|-------------------|
| F1 | 40 | 81 | 22 | 2.5x10 ⁷ | 92.6 |
| F2 | 39 | 110 | 14 | 4.0x10 ⁷ | 92.5 |
| F3 | 64 | 75 | 26 | 4.0x10 ⁷ | 92.6 |

Blood flow at the injection site evaluated by transvaginal enhanced ultrasonography



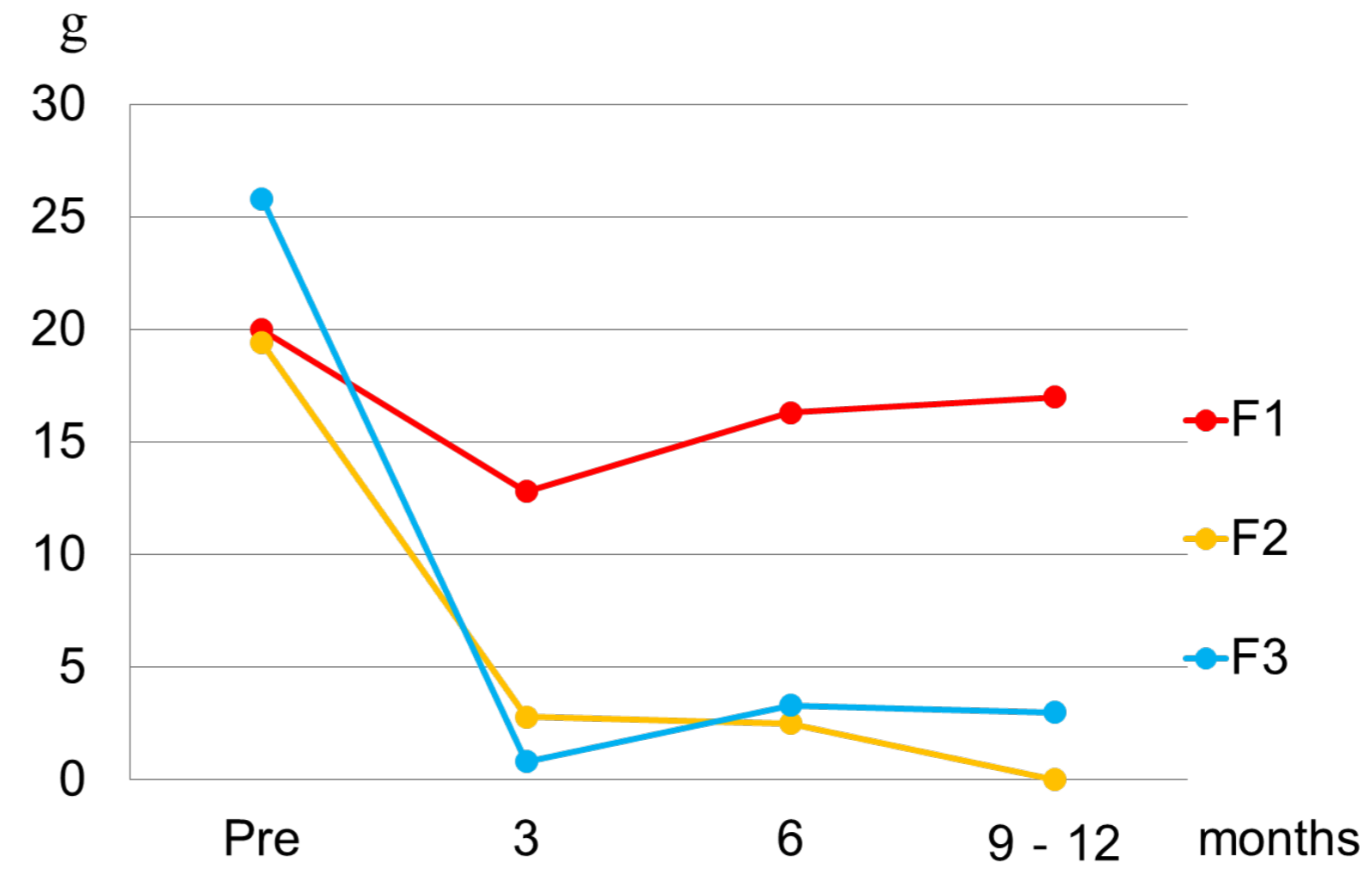
The bladder and urethra was visualized as a sagittal section. The blood flow around the injected area visualized as orange color was progressively increased after the injection of ADRCs up to 12 months. (case F2)

Changes in injected adipose tissue on MRI



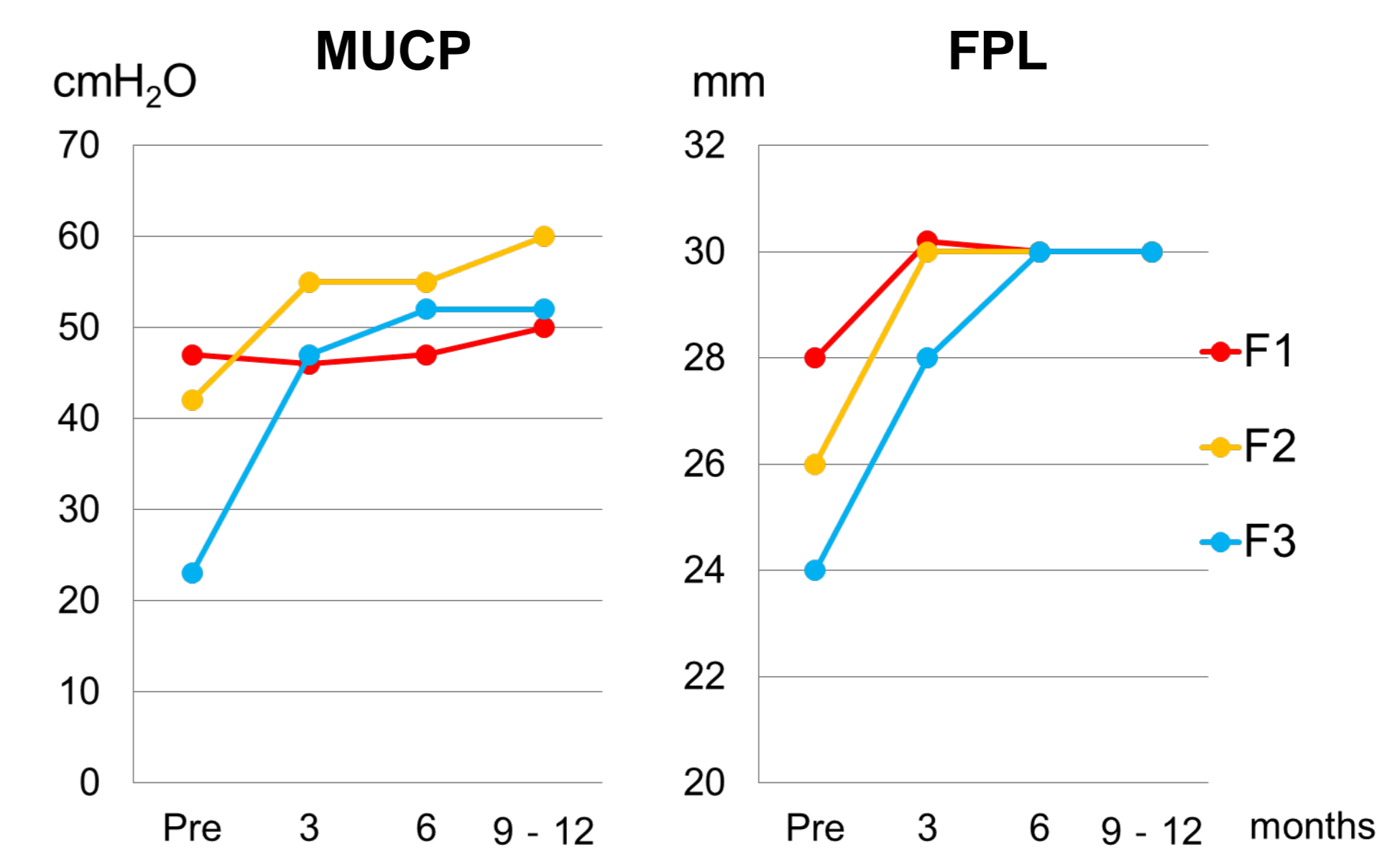
MRI demonstrated that injected fatty tissue remained at the injection site even 1 year after the injection (case F2).

Changes in leakage amount: 24-hour pad test



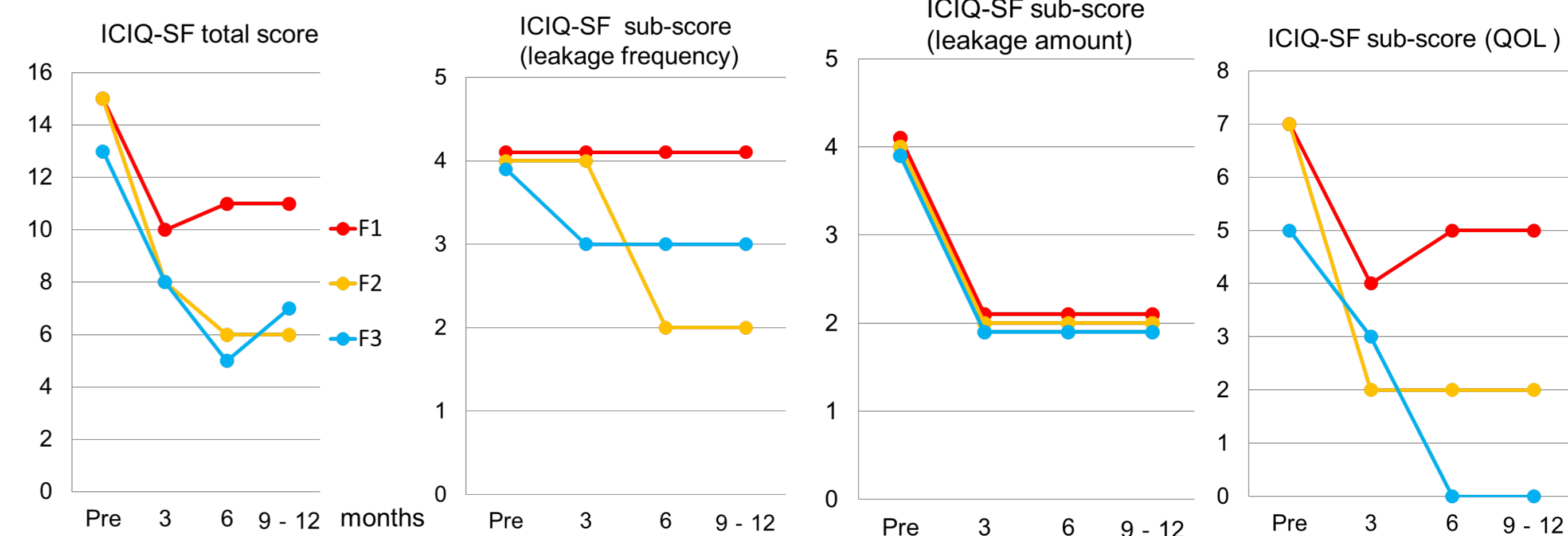
In 2 patients, leakage volume decreased progressively over time up to 12 months, no change in leakage volume was achieved in 1 case. One patient achieved total continence at 12 month after the treatment.

Changes in MUCP and FPL



MUCP progressively increased in 2 patients with improved leakage volume on the 24-pad test, but not in those with unimproved leakage volume. On the other hand, FPL increased in all patients.

Changes in Subjective Symptoms and QOL (ICIQ-SF)



In all patients, total score and QOL sub-scores of the improved. However, the patients without improvement of leakage amount showed no improvement in the ICIQ-SF sub-score for leakage frequency.

Voiding function

No patient had voiding dysfunction without residual urine at 6 and 3 months after the treatment.

Advantage

Our treatment strategy has an important advantage over the use of other stem cells.

1. Since adipose tissue contains abundant multipotent regenerative cells as well as key mature cells and progenitor cells, therapeutic levels of ADRCs can be obtained rapidly using the Celution™ system.
2. Unlike other cell therapy strategies, the treatment is all autologous, requires no cell culture and is performed in the context of a single surgical procedure.

Possible mechanisms for the ADRC-mediated improvement of the sphincteric function [2]

1. A bulking effect produced by the injected adipose tissue fraction mixed with ADRCs (Persistent bulking effect indicates the survival and growth of the injected adipose tissue, which could also be attributed to the presence of ADRCs).
2. Differentiation of injected cells into mature adipose tissue and possibly into contractile cells.
3. Increased blood flow due to angiogenesis effect of the cytokines secreted by the injected ADRCs. (The increased blood flow might have a positive effect on the regeneration of the injected adipose tissue and impaired intrinsic sphincter function).

[2] Watanabe T, Yamamoto T, Gotoh M, et al: Increased urethral resistance by periurethral injection of low serum cultured adipose-derived mesenchymal stromal cells in rats. Int J Urol, 18:659-666, 2011

Conclusions

The results of this preliminary study showed that periurethral injection of autologous ADRCs is a safe and feasible treatment modality for female patients with SUI caused by urethral sphincter deficiency.