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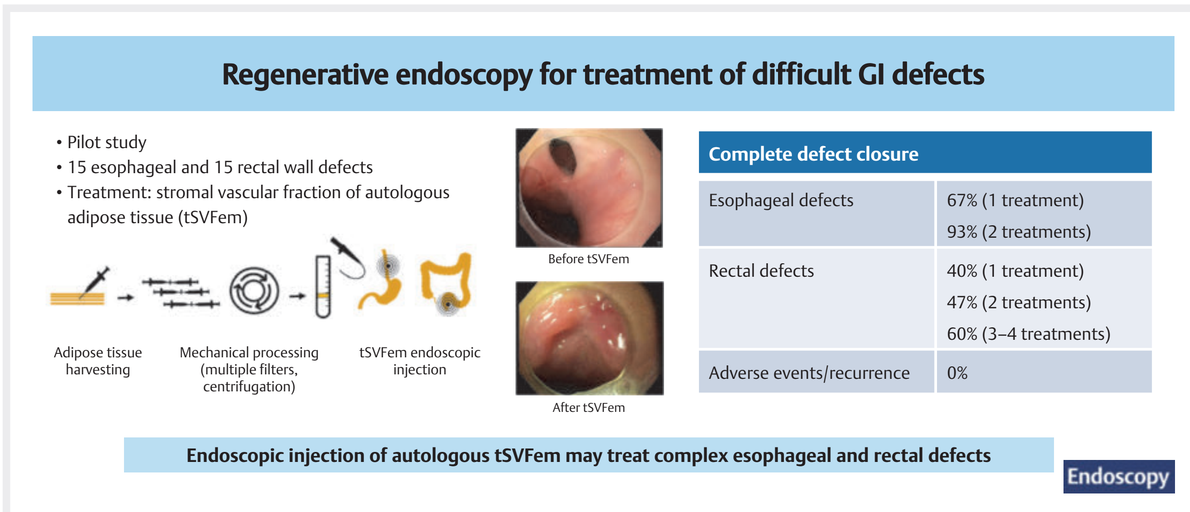
Regenerative endoscopy for the treatment of difficult gastrointestinal defects: results from a pilot trial

GRAPHICAL ABSTRACT



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ABSTRACT

Background Gastrointestinal (GI) defects with inflamed, fibrotic edges are often refractory to traditional endoscopic treatments. This study evaluates the efficacy and safety of endoscopically delivered stromal vascular fraction from autologous adipose tissue (tSVFem), which promotes tissue regeneration in upper and lower GI defects without additional patient risk or cost.

Methods This pilot trial involved patients with GI defects accessible by endoscopy after traditional treatments had failed. The tSVFem was derived from harvested hip fat, which was processed and injected endoscopically into the defect margins. The primary outcome was complete defect resolution. Secondary outcomes included treatment frequency, procedure-related adverse events, and recurrence.

Results 30 patients were included: 15 with esophageal defects (median diameter 6 mm) and 15 with rectal defects (median diameter 5 mm). Of the 15 esophageal defects, 14 showed complete resolution after tSVFem injection (10 after one injection and 4 after two injections). The overall resolution rate for rectal defects was 60% (six after one treatment, one after two treatments, and two after three or four treatments). The resolution rate was 5/9 for rectal defects communicating with the urinary tract and 4/6 for those communicating with other organs. No intraprocedural or postprocedural adverse events or defect recurrence occurred.

Conclusions These results suggest that endoscopic injection of autologous tSVFem may treat complex esophageal and rectal defects, including those communicating with adjacent organs other than the urinary tract.

Introduction

Gastrointestinal (GI) defects can result from various causes, including anastomotic leak after surgery, iatrogenic perforation, radiotherapy, or spontaneous perforation. These defects are associated with elevated mortality and morbidity, and require complex and multidisciplinary management [1, 2, 3]. To date, no standardized treatment has been established for GI defects. Treatment options may include conservative, endoscopic, or major surgical approaches depending on the patient's specific clinical characteristics. Chronic defects are challenging to treat owing to abundant fibrosis and the ineffectiveness of endoscopic direct suturing, stenting, and other endoscopic procedures. The idea of our treatment, based on the endoscopic delivery of stromal vascular fraction obtained by mechanical emulsification of autologous adipose tissue (tSVFem), is to regenerate and revitalize the fibrotic tissue will epithelial, stromal, and vascular cells [4]. The tSVFem comprises a heterogeneous population of stem and stromal cells (adipose tissue-derived stromal or stem cells) isolated from the perivascular and extracellular matrix of adipose tissue, and has anti-inflammatory and regenerative tissue-promoting effects, with no risk for the patient and no additional production costs, unlike enzymatic processes [5]. Indeed, the tSVFem is extremely rich in adipose-derived mesenchymal stromal cells, as confirmed by previous analysis conducted by our group based on immunohistochemistry and immunofluorescence (▶ **Fig. 1**).

This pilot study aimed to evaluate the effectiveness and results of endoscopic tSVFem injection for the treatment of esophageal and rectal defects.

Methods

Ethical statement

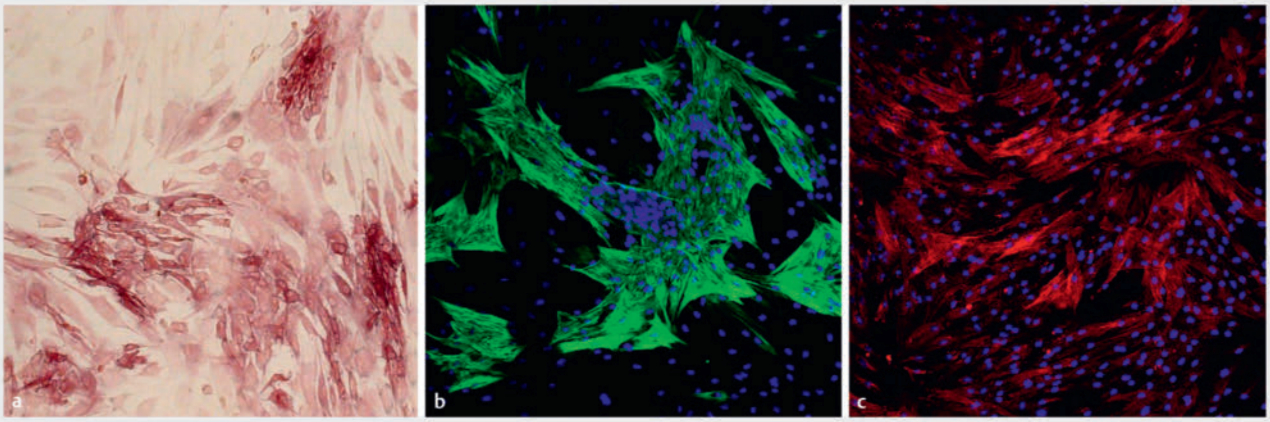
The study was approved by the Ethical Committee (Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy) in October 2020 (Prot. ID 3127), and conducted following the ethical standards of the Declaration of Helsinki and its later amendments. All patients involved in the study provided their informed consent, and their clinical data were treated anonymously. All authors had access to the study data, and reviewed and approved the final manuscript.

Study design

The study was a prospective, single-center pilot study. From October 2020 to June 2024, 30 consecutive patients with esophageal or rectal defects were treated by tSVFem injection. Clinical data were analyzed following the Strengthening the Reporting of Observational Studies (STROBE) checklist.

Inclusion criteria were GI defects accessible by endoscopy, for which all traditional conservative surgical treatments had failed or were excluded. Exclusion criteria were patients not providing consent to the study.

All esophageal procedures were performed with patients under general anesthesia and orotracheal intubation, while the rectal procedures were performed under deep sedation. The harvesting of the tSVFem and the operative endoscopy were performed simultaneously. The tSVFem was obtained by harvesting 30 mL of fat from the superficial layer of subcutaneous tissue of the patient. A 2.1-mm microcannula with four 1-mm



▶ **Fig. 1** immunohistochemistry and immunofluorescence analysis of stromal vascular fraction from autologous adipose tissue. **a** Alkaline phosphatase stain for human adipose-derived mesenchymal stromal cells. **b** Immunofluorescence analysis for the expression of smooth muscle actin (α-SMA) (green) to identify mesenchymal stromal cells in culture of dissociated adipose tissue; Hoechst was used to visualize nuclei (blue). **c** Immunofluorescence analysis for the expression of α-SMA (red) to identify mesenchymal stromal cells in culture of dissociated adipose tissue; Hoechst was used to visualize nuclei (blue).

holes (Trivisonno micro harvester cannula SuperLuer; Tulip Medical, San Diego, California, USA) was used to harvest the microfat [4, 6]. The tSVFem was obtained by centrifugation (3000 rounds per 3 minutes) after mechanical emulsification of the harvested microfat by sequential passages through 4.4 nm and 1.2 nm filters (Nanofat filter transfer set; Tulip Medical) and a 600/400-µm disposable filtering device (NanoTranfer; Tulip Medical) [4].

Endoscopy was performed to inject 1–2 mL of tSVFem into the submucosa of the four quadrants of the defect borders using a 20–22-G endoscopic needle to obtain complete defect closure. Further details of the technique are provided in a previous publication from our group [4].

Approach to large defects

Considering that direct endoscopic suturing of large chronic defects is as ineffective as surgery, and this is due mainly to abundant fibrosis, we defined larger diameter defects as those having a diameter of ≥5 mm. If technically feasible according to the endoscopist, these defects were sutured prior to injection with the purpose of approximating the margins rather than completely closing the defect. Endoscopic suturing was performed using the Overstitch suturing system (Boston Scientific, Marlborough, Massachusetts, USA).

Primary and secondary outcomes

The main study outcome was complete defect resolution at endoscopic and/or radiologic follow-up. Secondary outcomes were the number of days necessary for resolution, the number of treatments required, any complications related to the procedure, and any recurrences 60 days after resolution.

Sample size

The trial was designed as a pilot study. Given the rarity of the disease and considering Julious's statement for pilot studies, the sample size was set at 30 patients [7].

Statistical analysis

The results are expressed using descriptive statistics. Categorical variables are presented as numbers and percentages, and continuous variables are expressed as medians and ranges. Statistical analysis was performed using SPSS Statistics for Macintosh, version 25.00 (IBM Corp., Armonk, New York, USA).

Results

Of the 30 patients included in the analysis, 15 had esophageal defects and 15 had rectal defects. ▶ **Table 1** summarizes the patients' baseline characteristics and ▶ **Table 2** summarizes the outcomes, which are further described in **Table 1s** in the online-only Supplementary Material.

In 10/15 esophageal cases, complete defect resolution was achieved after the first treatment of tSVFem injection (▶ **Fig. 2a–d**). In five of these cases, suturing was performed to approximate the defect margins prior to injection of the stromal tissue into the submucosa. The five remaining patients underwent a second procedure after a median of 41 (range 20–60) days. At 60 days' follow-up from the last tSVFem injection, 14/15 patients (93.3%; 95%CI 68%–100%) showed complete defect closure, covered by new vascularized mucosa. Only one patient showed persistence of a 3-mm orifice after two tSVFem injections; however, the patient had undergone resection of a large esophageal diverticulum, resulting in a large orifice (12 mm) on the mechanical liner suture of the distal esophagus.

After the first tSVFem injection, the resolution rate for rectal defects was 40% (6/15) (▶ **Fig. 2e,f**). Concomitant endoscopic suturing was performed during the initial tSVFem injection in 10 patients with defects ≥5 mm (▶ **Fig. 2g,h**). The nine remaining patients underwent a second tSVFem injection procedure after a median of 82 (range 34–160) days. At 60 days' follow-up, 7/15 defects showed complete defect resolution. The

► **Table 1** Baseline characteristics of patients undergoing endoscopic injection of stromal vascular fraction from autologous adipose tissue for resolution of gastrointestinal defects.

	Esophageal defects	Rectal defects
Patients, n	15	15
Sex, male, n (%)	11 (73.3)	13 (86.7)
Age, median (range), years	47 (26–76)	65 (18–86)
Location, n (%)	Cervical: 2 (13.3)	Rectum-bladder: 8 (53.3)
	Middle esophagus: 3 (20.0)	Rectum-vagina: 2 (13.3)
	Distal esophagus: 7 (46.7)	Rectum-cutaneous: 2 (13.3)
	Cardia: 3 (20.0)	Rectum-urethra: 1 (6.7)
Cause, n (%)		
▪ Spontaneous	2 (13.3)	0 (0)
▪ Iatrogenic/traumatic	1 (6.7)	0 (0)
▪ Post-surgical	12 (80.0)	14 (93.3)
▪ Post-radiotherapy	0 (0)	1 (6.7)
Size, median (range), mm	6 (2–12)	5 (2–15)
Previous radiotherapy, n (%)	2 (13.3)	6 (40)
Previous chemotherapy, n (%)	5 (33.3)	6 (40)
Previous treatments for the GI defect, n (%)	6 (40.0)	7 (46.7)
▪ EVT	2 (13.3)	0 (0)
▪ SEMS	3 (20.0)	1 (6.7)
▪ OTS clip	0 (0)	1 (6.7)
▪ Surgery	1 (6.7)	5 (33.3)

EVT, endoscopic vacuum therapy; GI gastrointestinal; NA, not applicable; OTS, over-the-scope; SEMS, self-expandable metal stent.

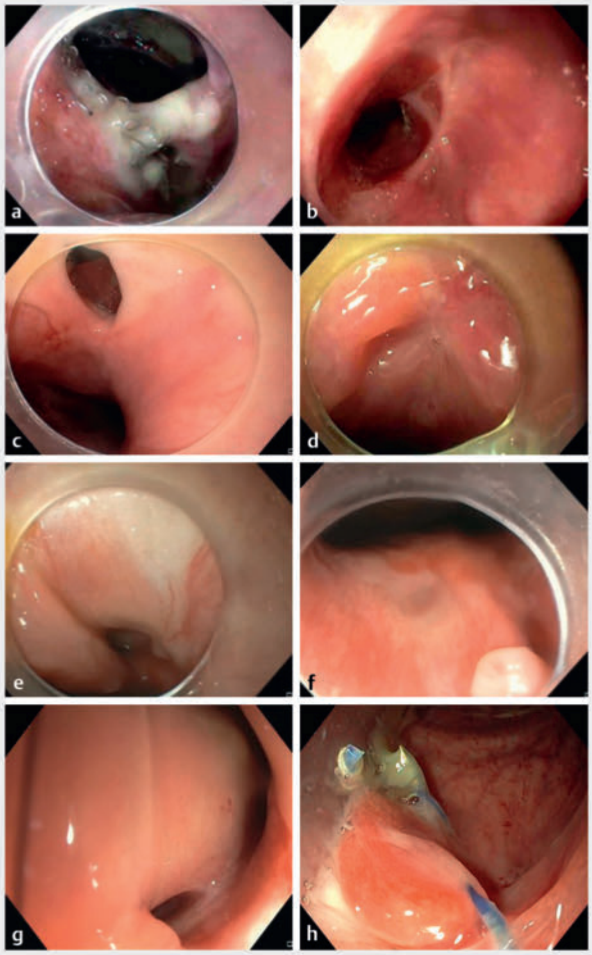
► **Table 2** Outcomes of endoscopic injection of stromal vascular fraction from autologous adipose tissue for resolution of gastrointestinal defects.

	Esophageal defects	Rectal defects
Time between acute event and treatment, median (range), days	90 (7–1800)	176 (21–2145)
Closure after 1 treatment, n (%) [95%CI]	10/15 (66.7) [41.9–91.0]	6/15 (40.0) [13–67]
▪ Endoscopic suturing, n/N (%)	5/15 (33.3)	10/15 (66.7)
Time between treatments, median (range), days	41 (20–60)	82 (34–160)
Closure after 2 treatments, n/N (%) [95%CI]	14/15 (93.3) [68–100]	7/15 (46.7) [21.0–73.0]
Closure after 3–4 treatments, n/N (%) [95%CI]	NA	9/15 (60.0) [32–84]
▪ Communication with the urinary tract		5/9 (55.6) [35–97]
▪ Communication with other organs		4/6 (66.7) [29–100]

NA, not applicable.

remaining eight patients underwent additional tSVFem treatment sessions. One case was resolved after the third session and another after the fourth, with an overall healing rate of 60% (9/15; 95%CI 32%–84%). The resolution rate was 55.6% (5/9; 95%CI 35%–97%) for defects communicating with the urinary tract and 66.7% (4/6; 95%CI 29%–100%) for the four defects communicating with other organs.

For both esophageal and rectal defects, the median procedure time was 45 (range 35–65) minutes. No intraprocedural



▶ **Fig. 2** Endoscopic images of gastrointestinal defects before (left panel) and after (right panel) endoscopic injection of stromal vascular fraction from autologous adipose tissue (tSVFem). **a,b** Postsurgical esophageal defect. **c,d** Esophageal defect. **e,f** Rectovaginal defect. **g,h** Rectovescical defect before and after tSVFem and endoscopic suturing.

or postprocedural complications, and no late complications or recurrence occurred during follow-up.

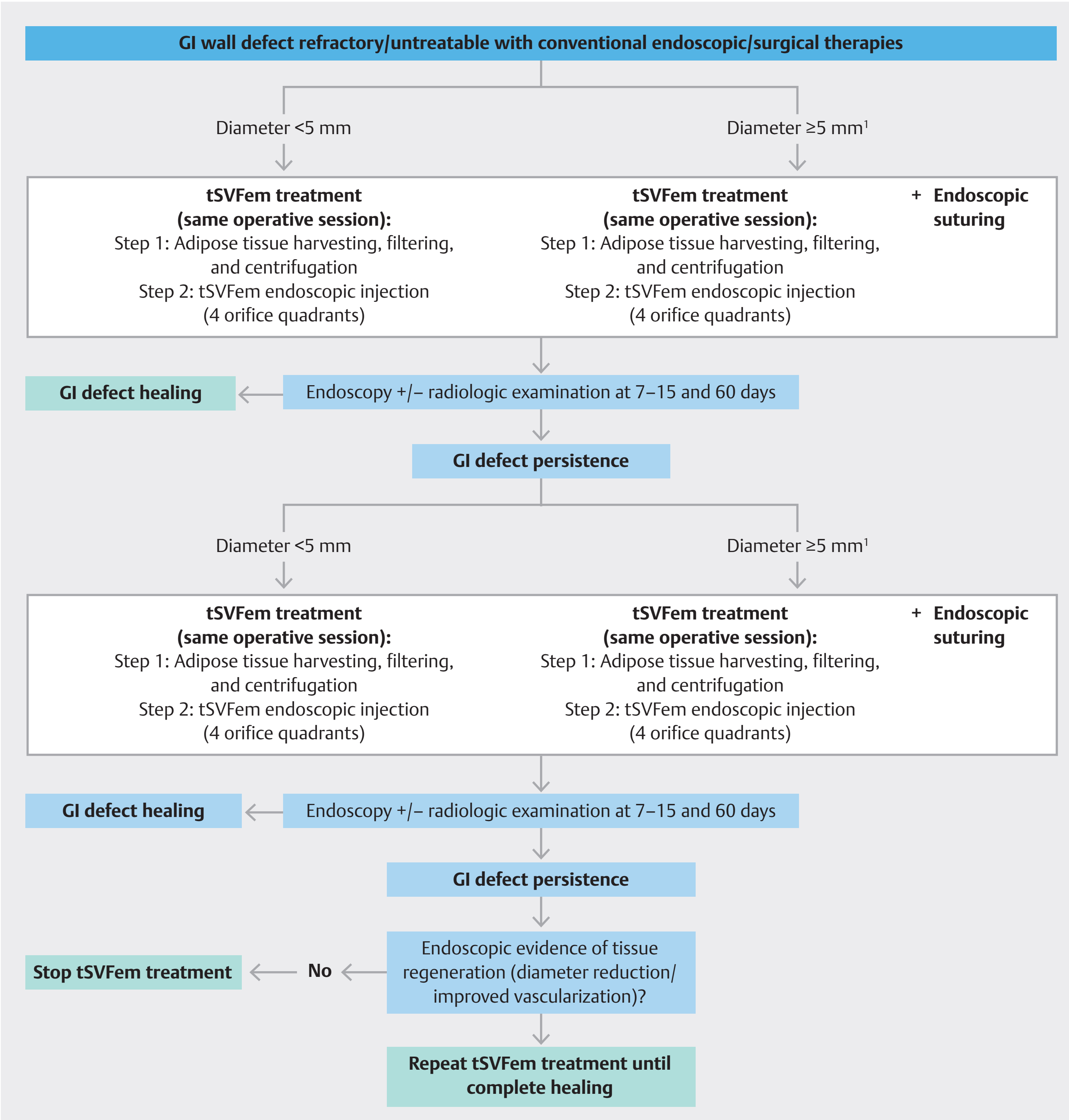
Discussion

Fat grafting and adipose-derived stem transplantation have applications in plastic surgery, orthopedics, maxillofacial surgery for joint regeneration and chronic pain, neurosurgery for neuropathic pain, and spinal cord injury [8, 9, 10, 11, 12]. Managing GI defects requires accurate diagnosis and a multidisciplinary approach based on clinical conditions, onset time, size, and anatomical characteristics. Several minimally invasive endoscopic techniques are available for treating GI defects, including endoscopic clips, endoscopic sutures, stents, and endoscopic vacuum therapy [2]. These techniques have limitations, including the need for long hospitalizations and advanced skill, complications, and significant costs [2, 13]. Most of these techniques fail to treat chronic defects with inflamed, fibrotic, retracted edges resistant to healing, even after tissue approximation [2]. This therapeutic challenge can be addressed using autologous tSVFem, which has regenerative tissue-promoting effects. Endoscopic delivery of autologous tSVFem, which contains mesenchymal stromal cells and extracellular matrix fragments, promotes healing through anti-inflammatory, proangiogenic, immunomodulatory, and regenerative processes [4, 5, 6].

Among the advantages of the tSVFem technique are its affordability and the absence of tissue rejection, as the grafted material is autologous. The tSVFem is obtained through mechanical tissue manipulation via sequential filters and centrifugation, without needing a cell factory or enzymatic agents. Furthermore, the collection, extraction, and endoscopic administration of tSVFem are performed during the same operative session [14]. From an endoscopic technical perspective, no advanced endoscopic skills are needed, as tSVFem delivery only requires the use of an injection needle [4, 5].

The current pilot study investigated the efficacy and safety of endoscopically delivered tSVFem for treating esophageal and rectal defects that were refractory or not amenable to traditional treatments. This technique resulted in 93.3% (14/15; 95%CI 68%–100%) complete esophageal defect resolution, with 10/15 cases resolved after only one session. The persistent defects after two sessions showed significant improvement, despite being very large. Endoscopic suturing was performed in one-third of cases to enhance tissue contact and promote healing. For rectal defects, the resolution rate after two sessions was 46.7% (7/15; 95%CI 21%–73%), with 6/15 resolved after the first session. The tSVFem injection included concomitant endoscopic suturing in about two-thirds of these cases. Despite this combination, complete healing remained suboptimal. However, improvement in diameter reduction, fibrosis reduction, and tissue tropism was observed, and two patients showed recovery after three and four sessions, respectively. Half of the patients had undergone previous radio/chemotherapy, a negative prognostic factor for defect closure [15]. The time between defect onset and tSVFem treatment was longer for rectal than esophageal defects (176 vs. 90 days). This "time effect" on tSVFem treatment efficacy may be due to the challenge of treating long-standing conditions. Multiple tSVFem treatment sessions may be the solution in some cases where tissue improvements are visible after the first two sessions (▶ **Fig. 3**).

A recent publication from our group demonstrated promising results of tSVFem injection for treating complex fistulizing perianal disease associated with inflammatory bowel disease, achieving 83% clinical healing. These results support the efficacy of tSVFem in promoting tissue regeneration [14]. Our investigation showed that defects communicating with the urinary system had lower healing rates (5/9) than those communicating with other organs (4/6). The reduced efficacy for urinary system defects is likely due to pressure variations between organs, pH differences, and bacterial colonization, which sustain inflammation. Our pilot study results suggest the need for improvement. Combining autologous tSVFem with 3D-printed tissue-engineered scaffolds may improve healing rates, and is currently under investigation (NCT06587633).



▶ **Fig. 3** Flow chart presenting the steps in the treatment of gastrointestinal defects using endoscopic injection of stromal vascular fraction from autologous adipose tissue (tSVFem). GI, gastrointestinal. [1] If available and technically feasible according to the endoscopist (location, tissue characteristics, maneuverability of suturing device).

As with every pilot study, this study is limited by the small sample size and the lack of a control group. However, the main limitation is the heterogeneity of cases regarding defect size, defect location, time of onset, etiology, and previous treatment, along with concomitant endoscopic suturing, which limits the interpretation of results and prevents us from performing an extensive analysis to predict factors associated with optimal or subclinical response. With regard to concomitant endoscopic suturing, it was used to approximate the mucosal edges and tissues, thereby facilitating the action of the tSVFem for larger defects when deemed feasible according to the endoscopist. The decision to perform suturing depended not only on the size of the defect but also on several other factors, including its anatomical location, the maneuverability of the endoscopic device, and the rigidity of the defect margins. These variables contribute to the challenges in standardizing the use of endoscopic suturing in conjunction with tSVFem therapy. As a general guideline, suturing should be considered for defects with a diameter of 5 mm or more, provided the suturing device is available and feasible based on the defect position, margin characteristics, and device handling (▶ **Fig. 3**). Furthermore, while the use of suturing undoubtedly increases the costs of the procedure, adds to the technical challenges associated with the procedure, and is not yet available in all departments, the technology for endoscopic suturing is advancing rapidly and is likely to become globally accessible in the near future [16]. Future research should also compare tSVFem with various other regenerative approaches, such as allogeneic mesenchymal stem cells and biomaterials, focusing on efficacy, safety, cost, and regulatory considerations. tSVFem offers regenerative factors with benefits in immune compatibility and a straightforward preparation method, free from complex regulatory burdens due to its minimal manipulation. In contrast, allogeneic mesenchymal stem cell approaches provide standardized preparation and the potential for large-scale production but carry a risk of immune rejection. Meanwhile, biomaterials offer structural support but lack cellular effects and may be more effective when combined with stem cell therapy [17]. Evaluating cost-effectiveness and long-term outcomes is crucial for determining the optimal clinical applications.

In conclusion, the proposed technique seemed effective for treating complex conditions, such as esophageal and rectal defects, especially for those communicating with organs other than the urinary tract. Considering these results, it is plausible to suggest that it could play a relevant role in managing difficult GI defects accessible by endoscopy, subject to further standardization of the technique and validation through larger prospective studies.

Acknowledgement

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Conflict of Interest

V. Bove is a consultant for Boston Scientific. C. Spada is a consultant for Medtronic and AnX Robotics, and has received speaker fees from Olympus and Pentax. I. Boskoski is a consultant for Apollo Endosurgery, Boston Scientific, Nitinotes, Pentax, Cook Medical, Microtech, ERBE, Siemens, Myka Labs, and Endo Tools Therapeutics S.A.; conducts sponsored lectures for Apollo Endosurgery, Boston Scientific, Cook Medical, and Microtech; is the recipient of research grants from Apollo Endosurgery, Endo Tool Therapeutics, and ERBE; and is a scientific advisory board member for Nitinotes and Myka labs. The remaining authors declare that they have no conflict of interest.

Clinical Trial

ClinicalTrials.gov | Registration number (trial ID): NCT04670276 | Type of study: Prospective

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