


## A clinical trial of autologous adipose-derived regenerative cell transplantation for a postoperative enterocutaneous fistula

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### Abstract

**Purpose** Adipose-derived stem cell (ADSC) transplantation is expected to be a minimally invasive, but effective, treatment for postoperative enterocutaneous fistulas associated with poor blood flow and chronic inflammation. The aim of this study was to assess the safety and efficacy of a novel ADSC therapy for this condition.

**Methods** We conducted an open-label, single-arm exploratory phase I study to assess the safety and efficacy of a novel ADSC therapy. Using the Celution system, we isolated adipose-derived regenerative cells (ADRCs) containing abundant ADSCs from liposuction-obtained gluteal adipose tissue. A mixture of ADRCs and fibrin glue was subsequently transplanted into the fistula, and ADRCs were percutaneously and endoscopically injected around the fistula. We evaluated the safety and feasibility of ADRC transplantation and fistula closure in six patients (UMIN000007316).

**Results** ADRC transplantation was completed in all patients. The fistula closure rates were 83.3 % at 4 and 12 weeks and 100 % at 24 weeks. All patients had grade 1 pain and subcutaneous hemorrhage at the liposuction sites, but no serious adverse events related to this procedure were observed.

**Conclusions** Transplantation of autologous ADRCs is safe, feasible and advantageous, as it can secure a sufficient cell count without culture or multiple passages, and will likely be effective for a postoperative enterocutaneous fistula.

**Keywords** Adipose-derived regenerative cells · Adipose-derived stem cells · Cell therapy · Enterocutaneous fistula

### Introduction

Gastrointestinal surgery can cause infectious complications at surgical sites, such as the body surface, organs, or body cavity, and anastomotic leakage in some cases [1–3]. To treat these complications, antibiotic administration, drainage, irrigation, and curettage are performed; decompression of the gastrointestinal tract using a drainage tube or ileostomy is also performed in some cases. Since these infections are often accompanied by a poor blood flow, achieving healing by unassisted tissue regeneration is difficult. Moreover, tissue restoration is often protracted in patients with concurrent inflammatory disease. The process results in a slow, long-term formation of a refractory fistula between the body cavity and the intestinal tract, markedly impairing the patient's quality of life (QOL) [4–6]. In several cases, resection of the diseased intestine with an enteric fistula and reconstruction should be considered and might be effective. However, some enterocutaneous fistulas secondary to diffuse peritonitis require abundant intestinal resection and might result in short bowel syndrome. Moreover, resection of the fistula related to anastomotic leakage at the lower rectum requires a permanent stoma. Thus, the resection of an enterocutaneous fistula is not always available in all patients. Approaches using tissue transplantation (e.g., omental flap or musculocutaneous flap transplantation) have been performed to treat enterocutaneous fistulas [7]; however, the surgery is invasive, and enterocutaneous fistulas often recur after tissue transplantation. Accordingly, tissue transplantation cannot be considered to be an established therapeutic method [8].

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Recently, tissue regenerative medicine for refractory diseases using stem cells has become more promising; however, ethical and safety hurdles regarding the use of embryonic stem cells (ES cells) derived from fertilized eggs and induced pluripotent stem cells (iPS cells) remain. Somatic stem cells play a primary role in tissue regeneration in organs such as the skin, intestinal epithelia, bone marrow, and blood by maintaining tissue homeostasis through constant cell division and differentiation. Although somatic cells do not have a differentiation ability as varied as that of ES cells and iPS cells, they are nevertheless being put to practical use in various fields. Mesenchymal stem cells are the most frequently clinically applied somatic stem cells. In addition to their differentiation ability, mesenchymal stem cells have angiogenic and immunoregulatory capabilities. Accordingly, they are attracting attention as a new therapeutic method [9]. While bone marrow-derived stem cells (BMDSC) have a rich history of use among mesenchymal stem cells, Zuk et al. reported in 2001 that adipose tissues also contain a large amount of mesenchymal stem cells [10]. Adipose-derived stem cells (ADSCs), which are mesenchymal stem cells like BMDSCs, have been revealed to possess pluripotent capability to differentiate into not only fat cells, but also cells of the cardiac muscle, bones, cartilages, nerves, and liver [9, 11–13]. Adipose tissues are more advantageous than bone marrow, as tissues can be harvested from patients in a less invasive manner. Similar to other mesenchymal stem cells, ADSCs have also been reported to have angiogenic and anti-inflammatory actions; thus, treatment applications for pathologies accompanied by blood flow disturbance and inflammation are expected [14, 15].

In this study, we demonstrated that treatment with ADSCs could be a novel, safe, and effective therapeutic method for refractory enterocutaneous fistulas.

## Methods

### Patients

The study included patients who had received, but not responded to, treatment such as antibiotic administration, drainage, irrigation, and ileostomy for an enterocutaneous fistula that developed after gastrointestinal surgery and persisted for at least 1 month. The fistula did not exceed 10 mL in volume and had to be evaluable with computed tomography (CT), magnetic resonance imaging (MRI), contrast imaging, or endoscopy. Other eligibility criteria included age between 20 and 75 years; body weight more than 40 kg; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; a normal cardiac function; adequate baseline bone marrow function (hemoglobin

more than 9.0 g/dL, white blood cell count more than 4000/mm<sup>3</sup>, and platelet count more than 100,000/mm<sup>3</sup>); an adequate hepatic function [serum total bilirubin (T. Bil) less than 1.5 mg/dL; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) less than 100 IU/L]; and an adequate renal function (serum creatinine less than 1.5 mg/dL).

The following were exclusion criteria: cancer or treatment with anticancer drugs; any gastrointestinal passage disorder; any active infectious disease; serious comorbidities (e.g., heart disease, lung disease, liver disease, renal disease, hemorrhagic tendency, inadequately controlled diabetes, or hypertension); oral anticoagulants or antiplatelets; a history of hypersensitivity to drugs; pregnancy, lactation, or the potential to become pregnant during the study. Written informed consent was obtained from all patients participating in this study.

### Treatment

Fat tissue specimens were harvested from the patients' gluteal subcutaneous tissues under general anesthesia in operating rooms. To suppress bleeding associated with harvesting fat tissue, epinephrine diluted to 0.25–1.5 mg/L was subcutaneously injected. In total, approximately 360 mL of fat tissue was harvested using standard sterile liposuction techniques. After harvesting, a concentrated ADRC solution containing a large amount of ADSCs was prepared using the Celution system (Cytori Therapeutics, San Diego, CA, USA). Briefly, adipose tissue was introduced into the Celution system, which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwarranted or deleterious cells and matrix fragments. Cells were counted using a Countess Automated Cell Counter (Invitrogen), and the average cell count was calculated. The release criteria included a cell count of  $3.5 \times 10^6$  or greater, viability of more than 60 %, and acceptable morphology. Phenotypic surface markers were also examined. Part of the concentrated ADRC solution was submitted for mycoplasma and bacterial testing, while part of it was frozen and stored.

After curettage and irrigation of the fistula, the concentrated ADRC solution was transplanted from the body surface and gastrointestinal tract. Using an injection needle, a fourth of the concentrated cell solution's volume was percutaneously injected into tissues around the fistula, and a fourth of the volume was endoscopically injected into the submucosal tissue around the fistula. The remaining half was mixed with thrombin solution and injected with fibrinogen solution (Bolheal; Kaketsuken, Kumamoto, Japan). Finally, the fistula was filled with fibrin glue containing ADRCs and the skin was sutured closed. Oral intake was resumed on the first postoperative day in except one patient

without ileostomy who experienced a transient exacerbation of Crohn's disease and therefore fasted for 20 days after the operation.

### Assessments

Patients underwent laboratory testing, fistulography, CT, and endoscopy at enrollment. Observations of the clinical symptoms, laboratory tests, CT results, and endoscopy assessments were performed at 1, 2, 4, 12, and 24 weeks after ADRC transplantation. Enterocutaneous fistula closure was considered to be achieved when fistula drainage disappeared, and no recurrence of an abscess or fistula was observed on the images. Upon diagnosis, the rate of enterocutaneous fistula closure was evaluated. All adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 4.0.

### Ethical considerations

This study's protocol was based on the Japanese guidelines for clinical research using human stem cells. The study was approved by the National Committee on Clinical Research Using Human Stem Cells and our institutional review board. This clinical trial was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (UMIN000007316).

### Results

#### Patient characteristics

The subjects included 4 men and 2 women (median age 36.5 years, range 27–55 years). The median BMI was 20.6 kg/m<sup>2</sup> (17.9–27.6 kg/m<sup>2</sup>). The primary diseases were ulcerative colitis in 4 patients (including 1 with a rectal carcinoid) and Crohn's disease in 2. One patient with rectal carcinoid and ulcerative colitis experienced moderate to severe colitis in the remaining colon while being treated for anastomotic leakage following low anterior resection for the rectal carcinoid. One patient with Crohn's disease experienced an enterocutaneous fistula extending from leakage at the anastomosis site to the drain insertion site following ileocecal resection. The other patient with Crohn's disease experienced an enterocutaneous fistula extending from the site of anastomotic leakage to the drain insertion site following ileocecal resection and anterior resection. Of 4 patients with ulcerative colitis, 3 had an enterocutaneous fistula extending from the pouch following total proctocolectomy and ileal pouch-anal anastomosis, and 1 had a fistula extending from the site of anastomotic leakage

following lower anterior resection for a rectal carcinoid. Prophylactic ileostomy was placed in one patient with ulcerative colitis after low anterior resection for the rectal carcinoid (Case 3). Diverting ileostomy was placed in 4 patients soon after the occurrence of enterocutaneous fistula (Cases 2, 4, 5 and 6), excluding 1 patient with Crohn's disease who had an enterocutaneous fistula extending from leakage at the anastomosis site following ileocecal resection to the drain insertion site. The median duration between the occurrences of enterocutaneous fistula and the transplantation of ADRCs was 23 months (5–44 months). The median fistula volume evaluated by fistulography was 1.5 mL (1.0–2.0 mL). The median level of preoperative C-reactive protein was 0.32 mg/dL (0.05–6.52 mg/dL). Although CRP was elevated up to 6.52 mg/dL in one patient complicated with a peristomal abscess (Case 2), there was no active inflammation in the fistularized intestine in all patients (Table 1).

#### Feasibility

All 6 patients successfully underwent fat tissue harvesting, concentrated ADRC solution preparation, and transplantation.

The median volume of the harvested fat tissue was 334 mL (range 290–359 mL). The subjects' body weight and BMI ranges were 45.9–75.5 kg and 17.9–27.6 kg/m<sup>2</sup>, respectively. Fat tissues required for treatment could be harvested in all patients.

The median ADRC value in the concentrated cell solutions was  $7.59 \times 10^7$  (range  $9.60 \times 10^6$ – $1.42 \times 10^8$ ), and the median viability of ADRCs was 89.2 % (range 84.3–93.0 %). The median percentage of CD45<sup>+</sup>CD31<sup>+</sup>CD34<sup>+</sup> cells was 24.2 % (range 1.9–49.7 %). No correlation was observed between the subjects' body weight or BMI and the amount of harvested fat tissues, ADRC count, ADRC viability, or the proportion of CD45<sup>+</sup>CD31<sup>+</sup>CD34<sup>+</sup> cells. One-quarter of the concentrated ADRC cell solution was percutaneously injected into tissues around the fistula, and one-quarter was endoscopically injected into submucosal tissue around the fistula. After the initial 2 injections, the remaining half of the ADRC solution was mixed with thrombin solution, injected into the fistula with fibrinogen solution, and the fistula was filled with fibrin glue containing ADRCs. The median operation time was 236.5 min (range 204–297 min), including the time to prepare the concentrated ADRC solution. The median time for liposuction and ADRCs transplantation was 68 min (range 59–105 min). The median blood loss was 20 mL (range 0–65 mL), including bleeding associated with curettage of the fistula. No complications associated with fat harvesting or transplantation were observed (Table 2).

**Table 1** Patient characteristics

Cases	1	2	3	4	5	6
Age (years)	27	42	43	31	55	29
Gender	Male	Female	Male	Female	Male	Male
Height (cm)	170	151	167	165	166	165
Weight (kg)	51.8	45.9	61.0	55.3	57.6	75.5
BMI (kg/m <sup>2</sup> )	17.9	20.1	22.0	20.4	20.8	27.6
Primary disease	CD	UC	Rectal carcinoid UC	CD	UC	UC
Previous operation	ICR	TP + IPAA	LAR	ICR + AR	TP + IPAA	TP + IPAA
Duration (months)	14	44	17	29	43	5
Therapeutic approach to enterocutaneous fistula	Drainage	Drainage + ileostomy	Drainage + ileostomy	Drainage + ileostomy	Drainage + ileostomy	Drainage + ileostomy
Fistula volume (mL)	2.0	1.0	1.5	2.0	1.5	1.5
Preoperative CRP (mg/dL)	0.47	6.52	0.12	0.16	0.05	1.24

CD Crohn's disease, UC ulcerative colitis, ICR ileocecal resection, TP total proctocolectomy, IPAA ileal pouch-anal anastomosis, LAR low anterior resection, AR anterior resection, CRP C-reactive protein

**Table 2** Separation, purification and transplantation of adipose-derived regenerative cells

Cases	1	2	3	4	5	6
Amount of fat removed by liposuction (mL)	290	333	335	359	324	355
Number of ADRCs	$1.16 \times 10^8$	$6.48 \times 10^7$	$5.20 \times 10^7$	$8.70 \times 10^7$	$9.60 \times 10^6$	$1.42 \times 10^8$
Viability of ADRCs (%)	93.0	88.8	84.3	88.3	89.6	92.1
Proportion of CD45 <sup>-</sup> CD31 <sup>-</sup> CD34 <sup>+</sup> Cells (%)	18.0	30.3	49.7	10.0	1.9	44.8
Filling with fibrin glue (mL)	2.5	2.5	2.5	2.5	2.5	2.5
Percutaneous injection (mL)	1.25	1.25	1.25	1.25	1.25	1.25
Submucosal injection (mL)	1.25	1.25	1.25	1.25	1.25	1.25
Total operation time (min)	206	248	250	225	297	204
Time for liposuction (min)	41	31	55	32	83	21
Time for ADRCs transplantation (min)	32	41	9	27	22	17
Blood loss (mL)	20	20	20	20	0	65

ADRCs adipose-derived regenerative cells

**Table 3** Clinical outcomes

Cases	1	2	3	4	5	6
Fistula closure						
At 4 weeks	+	+	+	+	+	-
At 12 weeks	+	+	+	+	+	-
At 24 weeks	+	+	+	+	+	+

### Efficacy

The fistula closure rates were 83.3 % (5 of 6 patients) at 4 and 12 weeks and 100 % (6 of 6) at 24 weeks (Table 3). Fistula closure and epithelialization were achieved in Cases 1–5 at 4, 12, and 24 weeks (final observation) after the transplantation of ADRCs. Case 1 resumed oral intake and

Cases 2–5 achieved ileostomy closure. At 4 and 12 weeks after ADRC transplantation, Case 6 had not achieved complete epithelialization despite decreases in fistula drainage; however, at the final observation 24 weeks after the procedure, drainage disappeared and epithelialization was observed. The patient had a recurrent fistula resulting from pouchitis relapse 10 months after the operation,

**Table 4** Adverse events

Cases	1	2	3	4	5	6
Liposuction-related pain	Grade 1	Grade 1	Grade 1	Grade 1	Grade 1	Grade 1
Liposuction-related subcutaneous hemorrhage	Grade 1	Grade 1	Grade 1	Grade 1	Grade 1	Grade 1
Transplantation-related pain	–	–	–	Grade 1	–	Grade 1
Fever	–	Grade 1	Grade 1	Grade 1	–	Grade 1
Dehydration	–	–	–	Grade 1	–	–
Vomiting	Grade 1	–	–	–	–	–
Diarrhea	–	–	–	–	–	Grade 1
Gastroesophageal reflux disease	–	Grade 2	–	–	–	–
Mucositis	Grade 1	–	–	–	–	–
Anal pain	–	–	Grade 1	–	–	–
Anal hemorrhage	–	–	–	–	Grade 1	–
Stoma complications	–	Grade 3	–	–	–	Grade 2
Hematuria	–	–	–	Grade 1	–	–
Maculopapular rash	–	–	–	Grade 1	–	–
Paronychia	–	–	Grade 1	–	–	–
Exacerbation of Crohn's disease	Grade 3	–	–	–	–	–
Anemia	Grade 3	Grade 3	–	–	–	Grade 1
AST	Grade 1	–	–	–	Grade 1	–
ALT	Grade 3	–	Grade 1	Grade 1	–	Grade 1
ALP	Grade 1	–	–	–	–	Grade 1
T.Bil	–	–	–	–	Grade 3	–
D.Bil	Grade 1	–	–	–	–	–
Hyponatremia	Grade 1	–	–	Grade 1	–	–
Hyperkalemia	–	–	–	–	Grade 2	–
Hypokalemia	Grade 1	–	–	–	–	–
Hypoproteinemia	Grade 1	–	–	–	–	–
Hypoalbuminemia	Grade 1	–	–	Grade 1	–	Grade 1
Hyperuricemia	Grade 1	Grade 2	–	Grade 1	–	Grade 1
Hypercreatininemia	Grade 1	–	–	Grade 1	–	–
Proteinuria	–	–	–	Grade 1	–	–
Increased levels of serum C-reactive protein	Grade 1	–	Grade 1	Grade 2	Grade 1	–

however, a small amount of drainage continued to the time of publication.

### Safety

Only 2 cases of serious adverse events were observed throughout each procedure stage, including the ADRC transplantation and observation period. The events included an extended hospitalization period resulting from exacerbation of Crohn's disease (Case 1) and hospitalization due to a parastomal hernia (Case 6). Grade 3 or greater adverse events were anemia in 33.3 % (2 of 6 patients) and stoma-related complications, increased ALT, and increased bilirubin in 16.7 % (1 of 6 patients) each; however, none of these adverse events were considered to be associated with fat tissue harvesting or ADRC transplantation. Although all patients experienced pain and subcutaneous hemorrhage at

the liposuction sites, all events were grade 1 and resolved or disappeared with the administration of analgesics (Table 4).

### Discussion

In this study, we demonstrated the advantages of transplanting autologous ADRCs containing a large amount of stem cells from the adipose tissues for treating postoperative enterocutaneous fistulas. This method can secure a sufficient number of cells without excessive culture or passage requirements and is a safe and feasible treatment for further consideration.

This study's strength lay primarily in its novel use of noncultured, nonpassaged ADSCs. Prior to clinical application, we verified the effect and mechanism of ADSC transplantation and demonstrated that ADSCs were involved in

angiogenesis, suggesting a potential mechanism for this study's therapeutic findings regarding fistula healing [16]. The limitations were the lack of a control group and a single-institution, nonrandomized design with a small sample size. Accordingly, while our results do not bear statistical significance, we believe the clinical results to be well worth further investigation on a large, multicenter scale.

The incidence of surgical site infection following colorectal cancer operation reportedly ranges from 10 to 30 %, and the incidences of anastomotic leakage are 3–6 % in colon cancer and 3–15 % in rectal cancer [17–22]. Despite declining rates of occurrence due to advances in the operative procedures and equipment, these complications are not rare [23]. In inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, the incidences of postoperative infectious complications such as anastomotic leakage are even higher due to preoperative malnutrition and the effect of immunoregulatory agents [24–27]. In addition, it is not uncommon for postoperative complications to lead to an enterocutaneous fistula in patients with Crohn's disease, for short bowel syndrome from polysurgery to result in any patient, or for pouch failure from pouch-related complication resulting in a permanent stoma to occur in patients with ulcerative colitis [5, 25]. Despite therapeutic techniques such as musculocutaneous flap surgery and filling using fibrin glue, to date there is no definitive, gold-standard therapeutic procedure. The development of a less invasive and highly effective therapeutic method thus remains desirable.

By treating fat tissues with enzymes and then centrifuging them, ADSCs, specific mesenchymal stem cells, can be fairly easily obtained. Fat tissues, which consist mostly of fat by volume, also contain vascular endothelial cells, vascular pericytes, macrophages, and ADSCs in their matrices [28]. After harvested fat tissues are treated with enzymes, these components, collectively known as the stromal vascular fraction (SVF), can be isolated by centrifugation. When the SVF is cultured, cells other than ADSCs die, establishing ADSCs as the primary component. Using multiple passages, even more cells can be obtained. There are other methods to secure mesenchymal cells; however, they tend to be less efficient. The ratio of the number of BMDSCs, mesenchymal stem cells like ADSCs, contained in the bone marrow is considered to be very low at 0.001–0.01 % of the total number of nucleated cells. Although it is not definite, harvested BMDSCs reportedly proliferate slower as they age following harvest [29]. On the other hand, approximately  $5 \times 10^3$  cells per gram of fat tissue can reportedly be harvested from ADSCs, 500 times greater than obtainable ADSCs from the same amount of bone marrow tissues [30]. Furthermore, large amounts of fat tissues are present near the body surface and can be harvested more easily and safely than bone marrow tissue. The clinical applications

are likely to favor the ability to secure a large number of ADSCs over relatively few BMDSCs. The clinical applications of ADSCs in various diseases are currently underway. If a sufficient amount of ADSCs can be initially secured through fat tissue harvesting and preparation, then cultivation and the use of multiple passages to increase the number of cells will become unnecessary, making it possible to provide treatment without a cell processing center, one of the current hurdles in cell therapy. This would be a highly convenient advantage. At present, the target diseases of treatment with ADSCs are wide-ranging, including fistulas, ischemic heart disease, peripheral artery disease, inflammatory bowel disease, urinary incontinence, and plastic reconstructive surgery [31–36]. Cultured or passaged ADSCs are used to treat some of these diseases [31, 32, 35]; in other cases, purified ADSCs with noncultured or passaged SVF are administered, as in this study, which used a Celution system [33, 34, 36]. The results of phase II and III studies of complex anal fistulas, pathologically similar to the enterocutaneous fistulas examined in this study, treated with cultured ADSCs have already been reported [31, 32]. Our novel study, based on our previous clinical findings of angiogenesis with ADSCs in experimental mice with intraperitoneal dead space, confirmed and expanded based on the results of the other fistula studies [16].

All 6 patients in this study had inflammatory bowel disease, ulcerative colitis, or Crohn's disease. Various treatment types were administered for enterocutaneous fistulas that had remained for more than a year. Local inflammation was controlled by drainage. Anti-TNF- $\alpha$  antibodies were administered to 2 patients with Crohn's disease and 1 patient with ulcerative colitis (after rectal carcinoid surgery), and intestinal tract inflammation was well controlled. Following ADRC transplantation, fistula closure was rapidly achieved in 5 patients. Case 6 required 4 months to achieve fistula closure, largely due to poorly controlled pouchitis after transplantation; 10 months later, an enterocutaneous fistula recurred from pouchitis relapse. Of 5 patients with temporary stoma placement, 4 patients experienced stoma closure. Case 6, with recurrent enterocutaneous fistula, did not experience stoma closure.

Using ADRC transplantation for refractory enterocutaneous fistula is thus considered to be a safe, novel therapeutic method and expected to be effective. The angiogenic influence we observed in our experimental mice is a likely contributing mechanism. Favorable fistula closure was achieved in patients with controlled intestinal tract inflammation, although complete fistula closure could not be achieved in patients with inadequately controlled pouchitis after transplantation. This result may indicate that the amount of ADSCs contained in ADRCs locally administered in this study was inadequate for the sustained control

of intestinal inflammation. Further study of ADSC inflammation control and immunoregulation is required.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

## References

- Golub R, Golub RW, Cantu R Jr, Stein HD. A multivariate analysis of factors contributing to leakage of intestinal anastomoses. *J Am Coll Surg*. 1997;184:364–72.
- Watanabe A, Kohnoe S, Shimabukuro R, Yamanaka T, Iso Y, Baba H, Higashi H, Orita H, Emi Y, Takahashi I, Korenaga D, Maehara Y. Risk factors associated with surgical site infection in upper and lower gastrointestinal surgery. *Surg Today*. 2008;38:404–12.
- Daams F, Wu Z, Lahaye MJ, Jeekel J, Lange JF. Prediction and diagnosis of colorectal anastomotic leakage: a systematic review of literature. *World J Gastrointest Surg*. 2014;6:14–26.
- Schwartz DA, Loftus EV Jr, Tremaine WJ, Panaccione R, Harnsen WS, Zinsmeister AR, Sandborn WJ. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology*. 2002;122:875–80.
- Fazio VW, Tekkis PP, Remzi F, Lavery IC, Manilich E, Connor J, Preen M, Delaney CP. Quantification of risk for pouch failure after ileal pouch anal anastomosis surgery. *Ann Surg*. 2003;238:605–14 (discussion 614–7).
- Sagar PM, Pemberton JH. Intraoperative, postoperative and reoperative problems with ileoanal pouches. *Br J Surg*. 2012;99:454–68.
- Oomen JW, Spauwen PH, Bleichrodt RP, van Goor H. Guideline proposal to reconstructive surgery for complex perineal sinus or rectal fistula. *Int J Colorectal Dis*. 2007;22:225–30.
- Giroto JA, Ko MJ, Redett R, Muehlberger T, Talamini M, Chang B. Closure of chronic abdominal wall defects: a long-term evaluation of the components separation method. *Ann Plast Surg*. 1999;42:385–94 (discussion 394–5).
- Nagaya N, Kangawa K, Itoh T, Iwase T, Murakami S, Miyahara Y, Fujii T, Uematsu M, Ohgushi H, Yamagishi M, Tokudome T, Mori H, Miyatake K, Kitamura S. Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. *Circulation*. 2005;112:1128–35.
- Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7:211–28.
- Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. *Trends Biotechnol*. 2006;24:150–4.
- Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM, Rice HE. Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun*. 2002;294:371–9.
- Rangappa S, Fen C, Lee EH, Bongso A, Sim EK. Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg*. 2003;75:775–9.
- Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004;109:1292–8.
- Puissant B, Barreau C, Bourin P, Clavel C, Corre J, Bousquet C, Taureau C, Cousin B, Abbal M, Laharrague P, Penicaud L, Casteilla L, Blancher A. Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *Br J Haematol*. 2005;129:118–29.
- Takahashi H, Haraguchi N, Nishikawa S, Miyazaki S, Suzuki Y, Mizushima T, Nishimura J, Takemasa I, Yamamoto H, Mimori K, Ishii H, Doki Y, Mori M. Biological and clinical availability of adipose-derived stem cells for pelvic dead space repair. *Stem Cells Transl Med*. 2012;1:803–10.
- Rullier E, Laurent C, Garrelon JL, Michel P, Saric J, Parneix M. Risk factors for anastomotic leakage after resection of rectal cancer. *Br J Surg*. 1998;85:355–8.
- Pryor KO, Fahey TJ 3rd, Lien CA, Goldstein PA. Surgical site infection and the routine use of perioperative hyperoxia in a general surgical population—a randomized controlled trial. *JAMA*. 2004;291:79–87.
- Yeh CY, Changchien CR, Wang JY, Chen JS, Chen HH, Chiang JM, Tang R. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg*. 2005;241:9–13.
- Itani KM, Wilson SE, Awad SS, Jensen EH, Finn TS, Abramson MA. Ertapenem versus cefotetan prophylaxis in elective colorectal surgery. *N Engl J Med*. 2006;355:2640–51.
- El-Gazzaz G, Geisler D, Hull T. Risk of clinical leak after laparoscopic versus open bowel anastomosis. *Surg Endosc*. 2010;24:1898–903.
- Fujita F, Torashima Y, Kuroki T, Eguchi S. The risk factors and predictive factors for anastomotic leakage after resection for colorectal cancer: reappraisal of the literature. *Surg Today*. 2014;44:1595–602.
- Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. *Best Pract Res Clin Gastroenterol*. 2004;18:865–80.
- Keighley MR, Eastwood D, Ambrose NS, Allan RN, Burdon DW. Incidence and microbiology of abdominal and pelvic abscess in Crohn's disease. *Gastroenterology*. 1982;83:1271–5.
- Elton C, Makin G, Hitos K, Cohen CR. Mortality, morbidity and functional outcome after ileorectal anastomosis. *Br J Surg*. 2003;90:59–65.
- Setti-Carraro P, Ritchie JK, Wilkinson KH, Nicholls RJ, Hawley PR. The first 10 years' experience of restorative proctocolectomy for ulcerative colitis. *Gut*. 1994;35:1070–5.
- Araki T, Okita Y, Uchino M, Ikeuchi H, Sasaki I, Funayama Y, Fukushima K, Futami K, Maeda K, Iiai T, Itabashi M, Hase K, Motoya S, Kitano A, Mizushima T, Maeda K, Kobayashi M, Mohri Y, Kusunoki M. Risk factors for surgical site infection in Japanese patients with ulcerative colitis: a multicenter prospective study. *Surg Today*. 2014;44:1072–8.
- Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, Sato K, Inoue K, Nagase T, Koshima I, Gonda K. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208:64–76.
- Mareschi K, Ferrero I, Rustichelli D, Aschero S, Gammaitoni L, Aglietta M, Madon E, Fagioli F. Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. *J Cell Biochem*. 2006;97:744–54.

30. Ohgushi H, Caplan AI. Stem cell technology and bioceramics: from cell to gene engineering. *J Biomed Mater Res.* 1999;48:913–27.
31. Garcia-Olmo D, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum.* 2009;52:79–86.
32. Herreros MD, Garcia-Arranz M, Guadalajara H, De-La-Quintana P, Garcia-Olmo D, FATT Collaborative Group. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula Advanced Therapy Trial 1) and long-term evaluation. *Dis Colon Rectum.* 2012;55:762–72.
33. Houtgraaf JH, den Dekker WK, van Dalen BM, Springeling T, de Jong R, van Geuns RJ, Geleijnse ML, Fernandez-Aviles F, Zijlstra F, Serruys PW, Duckers HJ. First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol.* 2012;59:539–40.
34. Marino G, Moraci M, Armenia E, Orabona C, Sergio R, De Sena G, Capuzzo V, Barbarisi M, Rosso F, Giordano G, Iovino F, Barbarisi A. Therapy with autologous adipose-derived regenerative cells for the care of chronic ulcer of lower limbs in patients with peripheral arterial disease. *J Surg Res.* 2013;185:36–44.
35. Kølle SF, Fischer-Nielsen A, Mathiasen AB, Elberg JJ, Oliveri RS, Glovinski PV, Kastrup J, Kirchhoff M, Rasmussen BS, Talmann ML, Thomsen C, Dickmeiss E, Drzewiecki KT. Enrichment of autologous fat grafts with ex vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet.* 2013;382:1113–20.
36. Gotoh M, Yamamoto T, Kato M, Majima T, Toriyama K, Kamei Y, Matsukawa Y, Hirakawa A, Funahashi Y. 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;21:294–300.