Surgical Innovation
1–8

DOI: 10.1177/1553350615572656
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Adipose Tissue–Derived Regenerative Cell–Enhanced Lipofilling for Treatment of Cryptoglandular Fistulae-in-Ano: The ALFA Technique

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Abstract

Background. The treatment of complex fistulae-in-ano is challenging and often includes a number of operations due to high rates of recurrence. Recently, techniques using in vitro expanded adipose tissue–derived stem cells have been described. We describe a novel treatment for cryptoglandular fistulae used in 7 patients, using a combination of surgical closure of the internal opening and real-time autologous adipose tissue–derived regenerative cells (ADRC)–enhanced lipofilling, without need for in vitro expansion. *Methods*. Following exclusion of active perianal sepsis, patients underwent a standard tumescent liposuction procedure, harvesting ~300 to 400 mL of raw lipoaspirate. The lipoaspirate was prepared in real time, using the Celution 800/CRS system to obtain the stromal vascular fraction containing ADRCs. After excision of the fistula tract and closure of the internal orifice, fresh ADRC-enhanced lipoaspirate was injected into and around the fistula tract. *Results*. At 6-months' follow-up, 5 of 7 (71.4%) patients showed clinical signs of fistula closure; one of these patients had a recurrence at 10 months due to sepsis. The remaining 4 patients (57.1%) all had complete fistula closure at a median of 46 months' follow-up. There were no adverse events associated with the technique, and no new incontinence. *Conclusion*. Treatment of cryptoglandular fistulae-in-ano with ADRC-enhanced lipofilling appears feasible and safe, and may add to the range of procedures that can be used to treat this difficult problem.

Keywords

fistula-in-ano, stem cells, adipose tissue–derived regenerative cells, surgery, lipofilling, colorectal surgery, tissue engineering

Background

"Complex" fistulae-in-ano are those fistulae that cannot be treated with a procedure resulting in the laying open of the fistulous tract, due to the risk of incontinence posed by the division of a substantial portion of the sphincter complex.[1] The most common etiologies of complex fistulae are cryptoglandular disease, perianal Crohn's disease, and iatrogenic causes. The aim of the surgical management of fistula-in-ano is to eradicate or obliterate the fistulous tract while maintaining the continence mechanism. Following initial treatment of the underlying sepsis and drainage of associated collections, frequently including the insertion of an inert rubber drain ("seton"), management strategies may include the debridement of the fistula tract and closure of the internal opening with a mucosal advancement flap, insertion of fibrin glue or fistula plugs to the fistulous tract, cutting setons or loose setons to establish long-term drainage. Occasionally, patients may even require the formation of a colostomy for fecal diversion, and some patients with severe perianal Crohn's disease ultimately undergo a proctectomy.

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The results of surgery to eradicate the fistula, however, have been relatively disappointing, with successful surgical closure rates between 55% and 86%[2-7] and successful fibrin glue treatment between 10% and 69%.[8-17] The initial results of studies using a collagen fistula plug were encouraging with closure of the fistula achieved in up to 87%,[15] but subsequent studies showed healing rates ~50% and less.[18-20] Several new techniques have been developed and show promising early results, such as the ligation of intersphincteric fistula tract (LIFT) procedure,[21] and video-assisted anal fistula treatment (VAAFT).[22]

Garcia-Olmo et al[23,24] combined fistula glue with adult adipose tissue–derived mesenchymal stem cells (ASCs) in a cell therapy–based approach, achieving initial long-term success in more than 70% of cases.[23,24] However, their technique required several weeks of in vitro enhancement cell cultures between cell harvest and application with a proportion of cultures lost to infection, and in a review of their long-term outcomes from the phase II trial, 58% of patients treated with ASC achieved long-term closure.[25] We describe a novel technique using real-time *a*utologous adipose tissue–derived regenerative cells (ADRC)–enhanced *l*ipofilling for *f*istula-in-*a*no (ALFA technique),without the need for in vitro expansion of the stem cell fraction, combined with surgical closure of the internal opening. Our results describe the early outcomes for 7 patients treated with the ALFA technique for complex cryptoglandular fistulae-in-ano.

Methods

Between December 2009 and March 2012, 7 patients with complex cryptoglandular fistulae-in-ano were treated within the remit of a feasibility study of an experimental procedure, approved and funded by the hospital's research and development department, and approved through the regional ethics committee. All patients had received one or more standard treatment attempts unsuccessfully prior to be enrolled in the study. Because of its nature, it was not possible to predict the anticipated time to fistula closure prior to commencement of the study, thus the primary aim focused on primary closure at 6 months; secondary aims included time-to-closure and recurrence rates at 12 months, although data for longer term follow-up is available to the investigators.

All patients were required to declare their consent for participation into the study, as well as to give consent for the combined lipoaspiration procedure and autologous cell transplantation including medical imaging for publication. Follow-up appointments were planned at 10 days, 4, 8, and 12 weeks, as well as at 6 and 12 months. Prior to surgery, presence of acute anorectal sepsis or infected collections were excluded by clinical and, as necessary, magnetic resonance imaging (MRI). The demographic and clinical details for all patients are outlined in Table 1; in all cases, at least 1 loose seton in the fistulous tract to maintain sepsis control and anatomy was present.

Liposuction

Following routine patient assessment for surgical fitness, a liposuction procedure using the tumescent technique with *manual* aspiration under local or general anesthesia is performed. Through 2 small bilateral flank incisions (0.5 cm), 1000 mL of normal saline solution with 2 mL epinephrine 1:1000, 50 mL of 1% lidocaine, and 1500 U of hyaluronidase are injected to allow tumescence of fat with minimal blood loss. A hollow blunt-tipped canula size 3 to 4 is moved rapidly back and forth through the anterior adipose compartment of the abdominal wall to disrupt the fatty stromal tissue and obtain approximately 300 to 400 mL of raw lipoaspirate. After completion of liposuction, the patient's abdomen is covered with a pressure dressing for 10 days to reduce hematoma formation.

Celution Technique

All but 50 mL of lipoaspirate is transferred into the tissue collection chamber of the Celution 800/CRS system (Figure 1; Cytori Therapeutics Inc, San Diego, CA, USA), washed to remove free blood and lipid, and then digested with the proprietary enzyme reagent Celase 835/CRS (Cytori Therapeutics Inc) to release the stromal vascular fraction (SVF) of the lipoaspirate. The SVF is then concentrated by short centrifugation and automated wash cycles to obtain the ADRCs, which are then taken out of the automated cycle of the Celution system and mixed with the supernatant adipose tissue fraction of the remaining lipoaspirate (ie, 5 mL of ADRC solution are mixed with 20-50 mL fresh lipoaspirate) depending on the estimated required volume for injection. The automated Celution process takes a total time of approximately 90 to 120 minutes.

Autologous Cell Transplant to Fistula-in-Ano

The patient is anesthetized and placed in lithotomy position. All fistulous tracts are identified and debrided. The internal opening(s) are closed with an intra-anal mucosal advancement flaps (Figure 2). The ADRC solution is injected employing a crisscross lattice technique into the fistulae and surrounding tissue to achieve maximal tissue density and filling of all adjacent tissue spaces (Figure 3). The external opening is obliterated by periorifice tissue bulking injection.

Postoperative Care

Postoperatively, patients are discharged from inpatient care as indicated, and are advised to keep the abdominal pressure dressing in place for 10 days when it is removed

Table 1. Patient Characteristics, Indications, and Follow-up Data.

	Sex	Age	Indication/Preoperative Finding	Injected Volume (mL)	Result and Follow-up
1	Male	49	Ischiorectal abscess 6 years ago, failed advancement flap, colostomy for 3 years, no comorbidity; MRI preoperative: High posterior trans-sphincteric tract just below left puborectalis muscle	55	Healed at 6 months; Stoma reversed at 10 months; To date, 53 months clinical follow-up: No recurrence or incontinence
2	Female	46	Ischiorectal abscess 3 years ago, COPD, WPW syndrome; clinically high left-lateral trans-sphincteric tract	25	Healed at 5 months; MRI (12 months): Complete fistula closure; To date, 49 months: No recurrence or incontinence
3	Male	55	Ischiorectal abscess 3 years ago, previous seton, minor soiling, no comorbidity; MRI preoperative: High right-posterolateral trans-sphincteric tract, external opening posteriorly	35	Healed at 5 months; MRI (10 months): Small posterior internal sinus without trans-sphincteric extension; To date, 46 months: No recurrence, persistence of minor soiling
4	Male	38	Ischiorectal abscess age 11, learning difficulties, partial laying open of perineal tract preoperatively; MRI preoperative: High anterior trans-levator tract	25	Healed at 2 months; MRI (6 months): Complete fistula closure; To date, 34 months: No recurrence or incontinence
5	Male	38	Left perineal abscess 1 year ago, morbid obesity; MRI preoperative: Left-anterolateral mid-trans-sphincteric tract	45	Failed to heal at 3 months; Subsequent treatment with cutting seton at 6 months; Resolution at 13 months: No recurrence or incontinence (34 months from ALFA)
6	Male	39	Right ischiorectal abscess requiring colostomy 1 year ago, no comorbidity; Clinically high right-lateral trans-sphincteric tract	25	Clinically healed at 6 months; Recurrence with contralateral horseshoe fistula extension at 10 months; Insertion of draining setons and subsequent advancement flap at 25 months; MRI (30 months): Fistula closure; Colostomy reversal at 33 months; Under active follow-up
7	Female	31	Labial abscess 1 years ago, recurrent deep vein thrombosis; MRI preoperative: elongated high anterior trans-sphincterandparalabial tract	30	Recurrence at 5 months; Partial laying open of perineal fistula and insertion of draining seton at 6 months; Mucosal advancement flap at 15 months: No recurrence or incontinence (28 months from ALFA)

Abbreviations: MRI, magnetic resonance imaging; COPD, chronic obstructive pulmonary disease; WPW, Wolf-Parkinson-White.

at first outpatient review. All patients are routinely prescribed laxatives and analgesia with paracetamol and other nonsteroidal anti-inflammatories. Patients are followed up at regular intervals thereafter as indicated, using clinical examination and MRI for assessment of progress.

Results

Between December 2009 and March 2012, 7 patients with complex cryptoglandular fistulae-in-ano were treated with autologous ADRC-enhanced lipofilling (Table 1). At 6 months, fistula closure was achieved in

Figure 1. The Celution 800/CRS system.

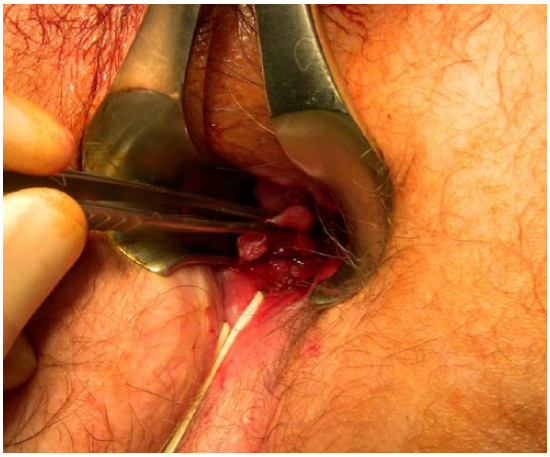

Figure 2. Closure of the internal fistula orifice with mucosal advancement flap.

5 patients (71.4%); however, 1 male patient (no. 6) showed initial closure of the external opening and complete cessation of discharge but developed recurrence with bilateral horseshoe fistulae and infected collections along both tracts after 10 months, requiring eradication of sepsis with bilateral seton insertion and subsequent

Figure 3. Adipose tissue–derived regenerative cells–enhanced lipofilling of the fistula tract and surrounding tissue.

successful myomucosal advancement flap with fistula core-out. Thus, complete fistula closure occurred in 4 cases (57.1%) with no recurrence at a median of 46 months' follow-up. The clinical result of successful treatment is shown in Figure 4.

One female patient (no. 7) with a long anterior tract and an external opening in the paralabial position showed partial healing over the first 5 months of follow-up with significant reduction in discharge and epithelialization of the external opening. However, because of ongoing sepsis within the lengthy tract, the fistula reopened thereafter and required subsequent partial laying open with shortening of the tract, enabling successful subsequent treatment with conventional advancement flap and fistula core-out. Only 1 patient showed no signs of fistula healing at any time in follow-up, a morbidly obese male patient (no. 5) with a body mass index of 44 kg/m^2 at the time of ADRC treatment, who since has been successfully treated with a tight cutting seton.

Safety

Liposuction was tolerated well by all patients, and there were no adverse events related to either anaesthetic or surgical intervention. No cases of incontinence were reported, only 1 male patient (no. 3) reported minor soiling of underwear that had been present also prior to the successful ALFA procedure. Both patients who had been treated with a temporary colostomy have had their bowel continuity successfully restored without incontinence.

Discussion

In recent years, advances in the field of regenerative medicine have made a large array of new treatments available

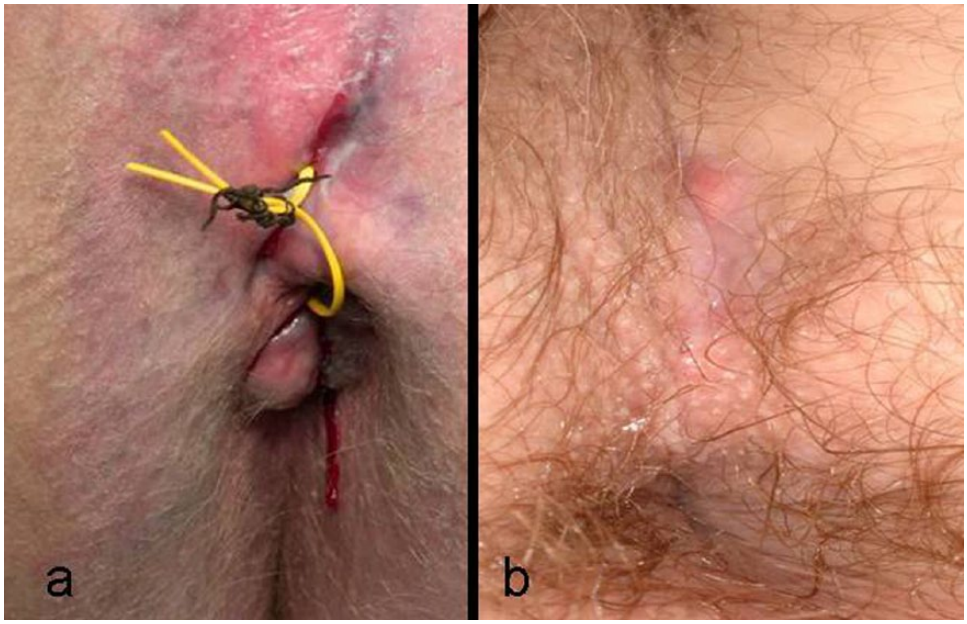

Figure 4. Preoperative appearance with seton in situ (a) and result at 4 month follow-up (b).

for the management of many medical and surgical conditions. Particularly the use of adult mesenchymal stem cells offers the possibility of extensive tissue renewal,[26] without the ethical and technical problems that are associated with the use of pluripotent embryonic stem cells.[27] While mesenchymal stem cells were initially harvested from bone marrow, it has since been shown that human adipose tissue contains a substantial proportion of so-called adipose tissue–derived stem cells (ASCs) and adipose tissue–derived regenerative cells (ADRCs),[28] with similar characteristics to bone-marrow derived mesenchymal stem cells. These similarities include their ability to differentiate into a large variety of cell types, including connective, endothelial, muscle, and scar tissue,[29] as well as substantial immunomodulatory properties.[30] There is increasing recognition that the efficacy of ADRCs, or stromal vascular fraction (SVF), is mediated through regulated release of growth factors by the therapeutic population rather than through differentiation into functional tissues. For example, in a study of chronic myocardial ischemia in rats, Premaratne et al[31] demonstrated that treatment with ADRCs led to reduction in the infarct size along with a statistically significant reduction in pro-inflammatory cytokines (interleukin 6 and tumor necrosis factor-α) and an increase in vascular endothelial growth factor. Similarly, Feng et al[32] showed that ADRC treatment led to a significantly reduced inflammatory response with reduced expression of certain pro-inflammatory growth factors in a rat model of acute renal ischaemia. Furthermore, both bone marrow–derived mesenchymal stem cells[33] and ADRCs[34] have been shown to improve cutaneous wound healing, with increased rates of epithelialization following treatment. In breast augmentation or reconstruction, graft retention is likely mediated by increased angiogenesis and stromal tissue regeneration.[35,36] Importantly, the benefits of ADRC treatment particularly are reported in ischemic and other hostile environment such as after radiation therapy.[37] In the case of fistulae-in-ano, a similar local reduction in inflammation along with promotion of angiogenesis is likely to improve the microenvironment for fistula healing.

For colorectal fistulating diseases, Garcia-Olmo et al[23] have described a technique combining fibrin glue with ASCs, with promising results of more than 70% initial success in a phase II randomized controlled trial, including both fistulating Crohn's disease and cryptoglandular fistulae.[24] However, the technique required in vitro cultivation of mesenchymal stem cells, a costly and time-consuming approach that poses limitation on its use in wider clinical practice. Indeed, in a subsequent phase III randomized controlled trial, conducted in several centers throughout Spain, the group confirmed favorable results for the ASC-enhanced fibrin glue *only* for the main author's pioneer centre.[38] In the context of perianal Crohn's disease, using direct injection of bone marrow–derived mesenchymal stem cells, Ciccocioppo et al[39] have achieved not only local success but also attenuation of the systemic inflammation. Only recently, Cho et al[40]

reported a series of 10 patients who were treated with ASC infiltration for Crohn's fistulae; of a total of 9 treated cases, the successful complete fistula closure rate was 4/9 (44.4%).

In the technique reported in this article, we used the automated process offered by the Celution system to obtain the SVF of the lipoaspirate. The cells of the SVF are available immediately for autotransplantation; it is estimated that ADRCs amount to approximately 1% of all nucleated cells in the lipoaspirate, more than 100-fold the yield obtained from bone marrow aspiration.[41] The Celution system is a closed system using single-use disposables, thus significantly reducing the risk of contamination posed by a lengthy process of in vitro cell culture. The results obtained in this feasibility study confirm that the technique is feasible and safe, as no adverse events occurred. The outcome of the ALFA technique is comparable to that described for the ASC/fibrin glue approach used in the pioneering Spanish trials[24,25] and to the results in Crohn's fistulae.[39,40] In 2 of our patients, persistence of focal sepsis is likely to have contributed to the failure of the technique; thus sepsis should be vigorously ruled out prior to ALFA treatment.

While the Celution process is comparatively more costly than conventional treatment options, it is significantly cheaper and less time-consuming than the treatments requiring in vitro expansion of ASC populations. We estimate the overall cost for 1 fistula treatment with ALFA is at around £4500.00 (€5400.00/US$7100.00) within the UK National Health Service, with £1450.00 (€1750.00/US$2300.00) attributable to the additional disposables required for the individual treatment. The capital cost for the Celution device in the United Kingdom is approximately £75 000 (€90 000.00/US$118 000), but the increasing availability of the Celution system for plastic and reconstructive surgery as well as cardiology, and the potential long-term benefit and cost savings for patients who otherwise frequently require additional subsequent treatments are likely to offset some of these costs.

One of the potential drawbacks of the presented data is the potential effect of the advancement flap; in general surgical practice and from observational data, its success is thought to be highly variable and may not exceed 60%[42] because of ischemia or infection. Thus, support of the advancement flap by biologically active material to enhance wound healing may be of additional benefit, as postulated by van der Hagen et al,[43] who successfully used platelet-rich plasma as adjunct for advancement flap repairof complex fistulae-in-ano. The technique of autologous ADRC-enhanced lipofilling in combination with surgical closure of the internal opening in patients with cryptoglandular fistulae-in-ano (ALFA technique) appears to be safe and effective, but further research in more patients, including those with Crohn's disease, and a direct comparison with conventional surgical techniques is warranted.

Author Contributions

DWB and PB developed the technique and the feasibility study. DWB, TSG, AKA and PB took part in the operative interventions. TSG, AKA, DKG and MAT contributed patients and undertook previous and preparatory procedures on thesepatients. DWB performed data collection, analysis and writing of the manuscript.

Authors' Note

The technique and associated data have been presented as oral poster presentation at the annual scientific meeting of the European Society of Coloproctology (ESCP), September 2012, Vienna, Austria, as poster at the annual scientific meeting of the Association of Coloproctology of Great Britain and Ireland (ACPGBI), July 2013, Liverpool, and as oral presentation at the Cell Society Europe 2013 Meeting, November 2013, Marseille, France and the Annual Meeting of the Spanish Association of Coloproctology 2014 Meeting, May 2014, Madrid, Spain.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: DWB and PB received training support in the ADRC harvesting and processing technique by Cytori Therapeutics Inc (San Diego, CA, USA). DWB received travel reimbursement from Cytori Therapeutics for the presentation of the technique and data, but no additional financial benefits direct from the company. DWB is Cytori Therapeutics Inc shareholder.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by the Research and Development Department of North Tees and Hartlepool NHS Foundation Trust, and ethical approval was granted by the Regional Ethics Committee, Sunderland, United Kingdom.

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