REVIEW

Modern management of anal fistula

Elsa Limura, Pasquale Giordano

Elsa Limura, Pasquale Giordano, Department of Colorectal Surgery, Whipps Cross Hospital, Barts Health NHS Trust, London E11 1NR, United Kingdom

Author contributions: Limura E acquisition of data, management of the database, data analysis and interpretation, drafting and revising; Giordano P conception and design, drafting and revising.

Correspondence to: Pasquale Giordano, MD FRCS, Department of Colorectal Surgery, Whipps Cross Hospital, Barts Health NHS Trust, Whipps Cross road, London E11 1NR, United Kingdom. p.giordano@londoncolorectal.org
Telephone: +44-20-85356656
Fax: +44-20-85356656

Abstract

Ideal surgical treatment for anal fistula should aim to eradicate sepsis and promote healing of the tract, whilst preserving the sphincters and the mechanism of continence. For the simple and most distal fistulae, conventional surgical options such as laying open of the fistula tract seem to be relatively safe and therefore, well accepted in clinical practise. However, for the more complex fistulae where a significant proportion of the anal sphincter is involved, great concern remains about damaging the sphincter and subsequent poor functional outcome, which is quite inevitable following conventional surgical treatment. For this reason, over the last two decades, many sphincter-preserving procedures for the treatment of anal fistula have been introduced with the common goal of minimising the injury to the anal sphincters and preserving optimal function. Among them, the ligation of intersphincteric fistula tract procedure appears to be safe and effective and may be routinely considered for complex anal fistula. Another technique, the anal fistula plug, derived from porcine small intestinal submucosa, is safe but modestly effective in long-term follow-up, with success rates varying from 24%-88%. The failure rate may be due to its extrusion from the fistula tract. To obviate that, a new designed plug (GORE BioA®) was introduced, but long term data regarding its efficacy are scant. Fibrin glue showed poor and variable healing rate (14%-74%). FiLaC and video-assisted anal fistula treatment procedures, respectively using laser and electrode energy, are expensive and yet to be thoroughly assessed in clinical practise. Recently, a therapy using autologous adipose-derived stem cells has been described. Their properties of regenerating tissues and suppressing inflammatory response must be better investigated on anal fistulae, and studies remain in progress. The aim of this present article is to review the pertinent literature, describing the advantages and limitations of new sphincter-preserving techniques.

Core tip: We present a review with a critical appraisal on the modern procedures for anal fistula which aims to the common goal of minimising the injury to the anal sphincters whilst preserving optimal function. We found the following ones as the most representative: Ligation of intersphincteric fistula tract, anal fistula plug derived from porcine small intestinal submucosa and the new designed GORE BioA® plug, fibrin glue, fistula

laser closure, video-assisted anal fistula treatment and adipose-derived stem cells. We examined the advantages and drawbacks for each procedure through the outcomes reported in literature.

INTRODUCTION

The ideal surgical treatment for anal fistula should eradicate sepsis and promote healing of the tract, whilst preserving the sphincters and the mechanism of continence. For the simple and most distal fistulae, conventional surgical treatment such as lay-open of the fistula tract as a complete transection of the tissue between the fistula tract and anoderm is very effective with a success rate of up to 100%[1]. Although reported incontinence rates following fistula surgery is very variable and is influenced by many factors, incontinence rate after laying open of intersphincteric and distal fistulae seems to be under 10%[2].

However, the risk of potential damage to the anal sphincters and subsequent poor functional outcome remains in a large proportion of patients with high fistulae when the tract crosses more than 30%-50% of the external sphincter, and with recurrent or complex fistulae with multiple extensions or separate tracts. Women with anterior fistula or previous obstetric injury as well as patients with pre-existing incontinence or specific risks such as previous local irradiation or co-existing Crohn's disease[3,4] are also at significant risk of incontinence and poor outcome. In these circumstances, an endorectal advancement flap (ERAF) which avoids the division of the sphincter complex is considered a safer alternative. However, the reported success rate is widely variable, ranging from 24% to 100%. Further, functional outcome assessments, in terms of post-operative incontinence rates, have been described as high as 35%[5-10].

The oldest surgical approach, first detailed by Hippocrates, is the use of a seton, which enables preservation of the sphincter mechanism[11]. The seton placement has been advocated either loose, to control infection, or cutting through the sphincter muscle gradually or as a bridge between two separate partial fistulotomies[12,13]. The short-term healing rate of loose seton ranges between 44% and 83%[14-18]. Cutting setons have been used in the attempt to slowly divide the sphincters while allowing scarring to occur and limit disruption of the muscular ring, with recurrence rates from 22% to 39%[19,20]. Regarding the comparison between seton and other fistulectomy, Quah *et al*[21] in a large multicenter Indian study (*n* = 503), showed longer healing with chemical setons but lower recurrence rate (4% *vs* 11%). Recently, a modified seton technique has been applied to treatment of trans-sphincteric and supra-sphincteric anal fistulas[22]. Recurrence rate was 0 in trans-sphincteric fistula and 4.9% in supra-sphincteric fistula. The technique provided favourable results in the treatment of complex anal fistula while preserving the sphincter function and conservation of faecal continence (0 incontinence rate).

Recently, a number of new sphincter-preserving techniques have been developed and proposed, all with the common goal of minimising the injury to the anal sphincters and optimise the functional outcome. However, the number of different procedures suggested, associated with a lack of follow-up data and the often variable and conflicting clinical outcomes has generated confusion and scepticism, resulting in a limited translation of these procedure into clinical practise.

The aim of this article is to provide an overview of the new sphincter-preserving techniques proposed for the treatment of fistula-in-ano, in particular assessing the efficacy and safety of these new procedures.

LITERATURE SEARCH

A search of the English-language literature was performed using PubMed, Cochrane, EMBASE and Ovid database to identify manuscripts reporting on the management of peri-anal fistula. The key-words used for this search included anal fistula treatments, fibrin glue, anal fistula plug, Ligation of the intersphincteric fistula tract (LIFT), expanded adipose-derived stem-cells (ASCs), video-assisted anal fistula treatment (VAAFT) and Radial-emitting laser probe (FiLaC™). Following articles of the available literature, 69 were selected as the most informative and recent publications. A further, more thorough review of these manuscripts was undertaken.

Available options of treatment

LIFT: Since it was first described by Rojanasakul *et al*[23] in 2006, LIFT gained popularity due to its high success rate and preservation of continence. This technique involves disconnection of the internal opening from the fistula tract at the level of the intersphincteric plane and removal of the residual infected glands, without dividing any part of the anal sphincter complex. Following the identification of the internal opening by injecting hydrogen peroxide from the external opening or gently probing the fistula tract, a 3-4 cm curvilinear incision along the anal margin is performed at the site of the fistula and the intersphincteric plane is entered. The fistula tract is exposed and ligated with two absorbable sutures. The tract is then divided distal to the point of ligation, with removal of the remnant of the fistula tract and any infected gland. Currette of the fistula tract and drainage of the external opening by an additional incision or an insertion of a catheter can also be performed. Re-approximation of the intersphincteric incision wound is achieved with interrupted absorbable sutures.

A recent metanalysis[24] over 24 original articles,

Table 1 Published articles on ligation of the intersphincteric fistula tract

Ref.	No. Patients	Follow-up (wk)	Healing rate	Complications	Type of study
Rojanasakul *et al*[23]	18	4	94%	NR	Prospective observational
Shanwani *et al*[26]	45	7	82%	NR	Prospective observational
Ellis *et al*[27]	31	6	94%	NR	Retrospective
Bleier *et al*[28]	39	10	57%	1 Anal fissure; 1 Persistent pain	Retrospective
Ooi *et al*[29]	25	6	96%	NR	Prospective observational
Tan *et al*[30]	93	4	92%	NR	Retrospective review
Steiner *et al*[31]	18	6	83%	1 hemorrhoidal thrombosis	Retrospective
Aboulian *et al*[32]	25	24	68%	2 Vaginal fungal infection	Retrospective review
Mushaya *et al*[33]	25	4	68%	1 Secondary bleeding; 2 Superficial perineal wound dehiscence	Prospective randomized
Abcarian *et al*[34]	50	15	74%	NR	Retrospective
Lo *et al*[35]	25	2	98%	NR	Retrospective
van Onkelen *et al*[36]	42	12	51%	NR	Prospective
Chen *et al*[37]	10	6	100%	NR	Retrospective
Lehmann *et al*[38]	17	4	47%	1 perianal haematoma; 1 wound infection	Prospective
Liu *et al*[39]	38	26	61%	NR	Retrospective

showed at a pooled mean 10-mo of follow-up, 76.5% mean success rate, 0 incontinence and and 5.5% post-operative complication rate. In Table 1, there are listed 14 articles[24,25] reporting on their experiences with LIFT procedure.

Of all the studies, only the Mushaya *et al*[33] one has a prospective randomized design, in which a group of 25 patients underwent LIFT and 14 advancement flap. Although, at 19 mo of follow-up, the recurrence rate was 7% (*n* = 1) in the ERAF group and 8% (*n* =2) in the LIFT group, the only patient with post-operative minor incontinence belonged to the advancement flap group. Furthermore, the operating time was shorter for LIFT procedures, as patients' return time to normal activities. As it emerges from the literature, 92.6% of all the patients treated, had a transphincteric fistula. The overall success rate ranges from 51% to 100% over a mean period of 2-24 wk. They report no postoperative impairment of continence or worsening of it, although it is not evaluated with a unique and different scoring system. Only 8 patients had minor postoperative complications (Table 1). Two studies[31,34] report a positive association between recurrence and the number of previous operations. This may be due to the presence of scar tissue that makes the identification of the intersphincteric tract more challenging. Indeed, in this procedure, an accurate dissection along the intersphicteric plane while maintaining the integrity of the internal sphincter is of vital importance for successful outcome. It is to be stressed the importance of an adequate drainage and elimination of any septic tissue *via* insertion of an indwelling seton for 6-12 wk prior to LIFT procedure. This allows a better outcome.

According to the literature, LIFT procedure has the advantages of preservation of the anal sphincters, minimal tissue injury, short healing time with no additional costs. In case of failure, the procedure can be readily repeated. However, to date, there is only one prospective-randomized trial and most of the evidence relies on small case-series with variable follow up and no objective assessment of incontinence.

In 2005, Ellis[27] introduced a variation of the LIFT technique in which a bioprosthetic graft was placed in the intersphincteric plane to reinforce the closure of the fistula tract (Bio-LIFT procedure). The bioprosthetic graft (Surgis Biodesign, Cook Surgical Inc, Bloomington, Indiana) was trimmed to the appropriate width and placed into the intersphincteric grove with at least 1 to 2 cm of overlap on all the sides of the transected fistula tract. The bioprosthesis was secured with 3/0 polyglicolic acid sutures to the levator ani muscle and the external sphincter to prevent migration. The fistula tract through the external sphincter was left open to facilitate drainage from the wound. Ellis treated 31 patients, all with transphincteric fistula. The overall healing rate was 94% after a minimum follow-up of 12 mo with no postoperative complications.

The only other description of the Bio-LIFT technique was for the management of rectovaginal fistulae[40] made by the same author. The bioprosthetic graft was deemed by the author to act as a physical barrier to separate the transected ends of the fistula.

However, we believe that a wider dissection into the intershincteric plane, the additional cost of the material and the higher learning-curve of this procedure, to date, is difficult to justify without any further supportive evidence.

Anal fistula plug

The anal fistula plug is a composed of lyophilized porcine-derived small intestinal submucosa [Surgis® anal fistula plug (AFP), Cook Biotech Incorporated, West Lafayette, Indiana, United States]. It is a strong pliable tissue denuded of cells, that provides a scaffold for host fibroblasts to promote tissue healing and repair damaged tissue. This material was initially intended for bridging large tissue defects in the abdominal and chest walls. Interestingly, a group of surgeons[41] rolled it into a cone

Table 2 Published studies on anal fistula plug

Ref.	Type of study	No. Patients	Success rate	Follow-up (mo)
Johnson *et al*[41]	Prospective Non-randomized Controlled trial	15	87%	7
Champagne *et al*[44]	Prospective	46	83%	12
O'Connor *et al*[45]	Prospective	20 (Crohn's)	80%	10
Van Koperen *et al*[46]	Prospective	17	42%	7
Ellis[47]	Retrospective	18 (5 rectovaginal)	88%	6
Lawes *et al*[48]	Retrospective	17 plug 3 pug+flap	66%	7.4
Ky *et al*[49]	Prospective	44 plug+flap	54.6%	6.5
Schwandner *et al*[50]	Prospective	19 (7 Crohn's)	61% overall 45.5% cryptoglandular 85.7% Crohn's	9
Garg[51]	Prospective	21	71%	10
Christoforidis *et al*[52]	Retrospective	47	43%	6.5
Thekkinkattil *et al*[53]	Prospective	43	44%	11
Safar *et al*[54]	Retrospective	35	14%	4
Ortiz *et al*[55]	Prospective Randomized trial	31 cryptoglandular: -15 plug -16 endorectal-advancement flap (EAF)	20% (plug group) 87.5% (EAF)	12
El-Gazzaz *et al*[56]	Retrospective	33	25%	7.4
Chan *et al*[57]	Prospective	44	50%	10.5
Cintron *et al*[58]	Prospective	73	42.5%	15

and inserted it into anal fistula in the attempt to achieve closure. Surgisis is a biocompatible material and has been documented to have an inherent resistance to infections in contaminated abdominal wounds in 2 series[42,43].

The technique of plug deployment is as follows: the tract is gently debrided with a curette and irrigated with hydrogen peroxide. The tapered end of the fistula plug is then tied to a probe and dragged through from the internal opening to the external. It is then pulled out to fit to the tract and anchored to the mucosa/submucosa and the internal sphincter at the primary opening with a "figure of 8" stitch to be eventually incorporated with the mucosa of anorectum and closure of the internal opening.

Johnson *et al*[41] prospectively compared 2 cohort group of patients undergoing plug procedure or fibrin glue closure. They reported an 87% of healing rate on the plug group, against 40% on the fibrin glue group. These initial results attracted significant interest in this technique, but such success rates have not been reproduced since (Table 2). Early implant extrusion has been reported as one of the most consistent reasons for failure[59]. It is also of crucial importance for such a procedure using biomaterials, that adequate removal of any chronic granulation or septic tissue lining the fistula tract is carried out, to initiate the healing process and allow migration of fibroblasts and endothelial cells. Another prospective randomized comparative trial was directed by Ortiz *et al*[55]. In this trial, 15 patients underwent plug insertion while 16 underwent an endorectal advancement flap (EAF) procedure. After 12 mo of follow-up, a large number of recurrences in the plug group (12 of 15 against 2 of 16 in EAF group) led to the premature closure of the trial. The literature reports a success rate ranging from 24% to 88% with a mean follow-up of 8 mo. A possible explanation for this discrepancy could be differences in patient selection and variation of the technique with respect to placement of the plug, bowel preparation, suture material, or coverage of the primary opening. For this reason, in an attempt to standardize the indications for use of bioprosthetic AFP and techniques for its placement, a consensus conference was held in 2007. According to that, the use of AFP should be recommended in transphincteric anal fistulae without any acute inflammation or infection. It was also suggested that a frequent issue affecting the AFP procedure, is a failure in technique of the plug placement[60].

Because of the fixation problem with the use of AFP, a new absorbable plug that could be better fixated, thanks to its design, has been introduced. The GORE BioA® fistula plug (W.L. GORE and Associated Flagstaff, Arizona, United States) is composed of a bio-absorbable monofilamentos compound with polyglycolic acid: trimethylene carbonate. Histological assessment confirms that the material is completely absorbed by 7 mo with no chronic inflammatory response evidence[61]. It is a 6 arms plug, of a length of 9 cm attached to a circular disc. The disc is meant to be sutured at the internal opening and, if necessary, some of the arms can be cut to fit to the fistula tract. After its disc is fixed to the internal opening by 2-3 absorbable stitches, a mucosa/submucosa flap, proximal to the internal opening, is raised to cover the suture.

To date, there are only 4 published studies assessing the GORE plug (Table 3). One of them retrospectively compared a series of patients treated with the Surgis AFP with those treated with GORE BioA® plug[61]. It reports that the patients treated with BioA® plug have

Table 3 Published studies on GORE BioA fistula plug

Ref.	Study design	No. patients	Aetiology and classification	Healing-rate	Follow-up (mo)	Complications
Buchberg *et al*[61]	Retrospective Case-series	10	6 cryptoglandular 3 iatrogenic 1 HIV	54%	2	NR
de la Portilla *et al*[62]	Prospective Case-series	19	10 high- transphincteric 9 low transphicteric	16%	12	1 Acute sepsis 1 Urge incontinence 1 Plug extrusion
Ratto *et al*[63]	Prospective Case-series	11	11 High- transphincteric	73%	5	NR
Ommer *et al*[64]	Retrospective Case-series	40	28 High-transphincteric 12 Suprasphincteric 4 Crohn's	57.5%	12	1 Plug extrusion 1 abscess 1 Dehiscence

higher initial clinical success rate and this seems to be due to less frequent implant extrusion rather than any biological advantage. The overall success rate, according to these studies, ranges from 16% to 73%, decreasing with the length of follow-up, which ranges from 2 to 12 mo. The highest number of patients among these studies, belongs to Ommer *et al*[64], where a group of 40 patients underwent GORE plug insertion, with a success rate of 57.5%. They reported higher healing rates in patients with transphicteric rather than suprasphincteric fistulae. No negative impact on anal continence was observed. The other studies are small case-series and all of them reporting heterogeneity of fistula aetiology and variability in terms of management of the primary tract and postoperative protocols. The cost, at €500, for the GORE plug is relatively high. Bigger cases series and comparative studies with other procedure are desirable, to further elucidate the cost-effectiveness of this technique.

Fibrin glue

Initial studies on fibrin glue injection for the management of complex anal fistulae were promising. The first of these was published in 1991 by Hjortrup *et al*[65] and was the result of a pioneering series of treatments for perianal fistulae with fibrin glue. The mode of action is thought to be by stimulating the growth of fibroblasts and pluripotent endothelial cells into the fistula tract to seal it off. These cells then lay collagen and an extracellular matrix during the process of wound healing. Early results were encouraging, but further data showed a very wide range of success from as low as 14%[66] to as high as 74%[67]. As many subsequent studies confirmed, long-term follow-up evaluation is very important when using fibrin glue because later follow-ups revealed the healing rates decrease markedly[68-70]. In their review, Swinscoe *et al*[68] noticed that, when fibrin glue was applied to complex fistulae in particular, it achieves a lower success rate (mean 70%) than simple fistulae (mean 32%). Furthermore, shorter fistulae (< 4 cm) tend to recur more frequently than longer ones (> 4 cm), with rates of 54% as compared with 11% respectively. A possible explanation is that shorter fistulae do not hold the glue as well as longer-tract fistulae do[71]. Despite the absence of impairment of normal continence as a

complication of this procedure, formation of abscesses and new secondary tracts have been reported with a incidence of up to 3%[69,72]. As Lindsey *et al*[72] showed in a randomized comparative trial, patients treated with fibrin glue didn't suffer any form of incontinence whilst the group treated with conventional surgical techniques did, but at post-operative MRI scan review, showed non-eradicated septic collections that potentially leads to secondary tracts.

Poor outcomes with fibrin glue may be explained with the failure of the formed glue clot to seal the tract properly, due to its liquid consistency[48]. Also, as Buchanan *et al*[66] reported, there may be formation of abscesses that lead to longer-term recurrences. Particularly, this event occurs when synthetic glue (cyanoacrylate glue, Glubran® 2, GEM S.R.L., Viareggio, Italy) is used. This glue occludes the fistula and degenerates into multiple abscesses around the glue remnants. It still has a role in reinforcing suture closure of the internal opening in other procedures such as VAAFT.

Fistula laser closure

The use of laser in the treatment of anal fistula was initially described in 2011 in a pilot study by Wilhelm[73]. This novel sphincter-saving technique uses an emitting laser probe [Fistula laser closure (FiLaC™), Biolitec, Germany], which destroys the fistula epithelium and simultaneously obliterates the remaining fistula tract. Since the main reason for surgical failure is a persistent fistula tract or remnants of fistula epithelium which were not excised, it was postulated that the benefit of this newly designed radial-emitting laser probe was to eliminate fistula epithelium or any granulation tissue in a circular manner and then, to obliterate the fistula tract by a shrinkage effect. Simple diathermy cannot elicit the shrinkage effect on tissues and it's more difficult to regulate its potential thermal damage on the sphincter muscles.

The procedure also includes the closure of the internal opening by means of an anorectal flap. When some scar tissue prevents that, either mucosa or anodermal flap is used for closure of the internal opening. In this pilot study, 11 patients with cryptoglandular fistulae, underwent FiLaC™ procedure with an overall success of 81% at 7, 4

mo of follow-up.

A modified laser procedure was adopted by Giamundo *el al*[74]. It consists of sealing the fistula tract by laser with no need for endorectal flap. The closure of the internal opening is allowed by a laser shrinkage effect.

The procedure in carried out similarly in both techniques, except from the performance of an advancement flap prior to the laser application. It is as follows: Identification and localization of the internal opening by the injection of hydrogen peroxide or methylene blue from the external opening. Debriding of the fistula tract with a curette. Insertion of a plastic hollow 14F catheter using a guide-wire. Insertion of 400 micron radial-emitting disposable laser fibre into the catheter with its tip emerging at the internal orifice. The fibre delivers laser energy homogenously at 360° and by applying continuous energy, the tract is closed while withdrawing it at a speed of 1 mm/s. Depending on the width of the tract, a 980 nm or 1470 nm diode laser, delivering different wavelengths, could be deployed. The first delivers 13 watts power, whereas the second 10 W.

Giamundo *et al*[74] performed FiLaC™ procedure on 35 patients with cryptoglandular and Crohn's disease-related fistulae. The overall success rate was 71% at 20 mo of follow-up. They didn't report any impairment of continence, but postoperative pain and anismus in 8 patients treated with the 980 nm diode laser. This may be due to the application of higher energy volumes. For this reason, the authors consider that the use of a 1479 nm diode laser for FiLaC™ is preferable to the 980 nm. They also suggest the use of a draining seton prior to the procedure, to help to create a more homogenous calibre of the tract and contribute to the closure on secondary tracts. Oztürk *et al*[75] reported a success rate of 82% at 12 mo of follow-up, on 50 patients treated for inter-sphincteric and ranssphinteric fistulas.

From these studies, it emerges that this procedure has some advantages. It doesn't affect continence, administers controlled hyperthermic effect to the tissues, has a short learning-curve and reduces postoperative hospital stay as compared to endorectal advancement flap or LIFT. However, it has also some drawbacks. It is a "blind" procedure, as it's not possible a direct visualization of the fistula tract or any secondary tracts and this may lead to recurrence. It requires expensive equipment, particularly if compared to other sphincter-saving techniques. Randomized trials comparing FiLaC™ with the most common sphincter-preserving procedure are therefore required.

Video-assisted anal fistula treatment

Video-assisted anal fistula treatment is performed with a kit which includes a fistuloscope manufactured by karl Storz GmbH (Tuttlingen, Germany), an obturator, an unipolar electrode, an endobrush and 0.5 mL of synthetic cyanoacrylate glue (Glubran® 2, GEM S.R.L., Viareggio, Italy). The fistuloscope has an 80 angled eyepiece and is equipped with an optical channel and also a working and irrigating channel. Its diameter is 3.3 mm × 4.7 mm and its operative length is 18 cm. The fistuloscope has 2 taps, one of which is connected to a 5000 mL bag of glycine-mannitol 1% solution, depending on the position of the fistula. This technique was developed by Meinero and Mori[76] in 2006 and his study is the only available to date. VAAFT consists of a diagnostic phase, followed by an operative phase. In the diagnostic phase, the fistuloscope is inserted through the external opening and advanced by the irrigation of the glycine-mannitol which expands the fistula tract. The fistuloscope, usually, exits through the internal opening. Once the internal opening is located, 2/3 stitches are placed to isolate without closing it. During the next operative phase, the aim is to destroy the fistula tract from the inside, curette it and close the internal opening. For this purpose, the electrode replaces the obturator from the external opening, under direct vision. The fistula wall is cauterized and all wasted material is eliminated into the rectum through the internal opening. Ultimately, the internal opening is sutured by a semicircular or linear stapler (roticulator). When the tissue around the internal opening is too thick to be stapled, a cutaneous or mucosal flap is fashioned. In order to reinforce the suture or staple line, 0.5 mL of synthetic cyanoacrylate glue can be applied.

In their study, 136 patients with non-Crohn'disease-related anal fistulae, within 2-3 mo of follow-up, achieved an overall success rate of 73.5%. No postoperative incontinence or its worsening was reported. We believe that the rational of this technique regarding the precise identification of the fistula tract is robust, and there are minimal postoperative wounds. However, it shouldn't be denied that in some cases, an excessive dilatation of the fistula to insert the fistuloscope, the risk of missing other secondary tracts or the internal opening itself and also, the risk of thermal damage by the electrode (which is higher than that of the laser) cannot justify the high cost of the kit, plus the stapler, a healing rate comparable to other procedure and, least of all, a long operative time. Therefore, improvements of the technique and further studies are required.

Adipose-derived stem cells

The potential role of mesenchymal adult stem cells in differentiating into various types of cells may have a role in the treatment of anal fistula. In a pilot trial, Garcia-Olmo *et al*[77,78] chose adipose ASCs. Application of autologus ASCs (Cx401) represents a novel approach for enhancing regeneration of damaged tissues in an environment particularly unfavourable for wound healing. ASCs can be obtained from subcutaneous fat[79,80] by liposuction and this process yields 100 times more stem cells than bone marrow-aspirates[81]. Following curettage of the fistula tract and suture closure of the internal opening, ASCs solution was injected into the tract and into the walls of the fistula. The tract was subsequently sealed with fibrin glue.

It was deemed that ASCs biological properties of suppressing inflammation and promoting differentiation

can accelerate wound healing. Two previous trials were conducted by the same authors: a concept phase I clinical trial in Crohn's disease[77] fistulae and a phase Ⅱ clinical trial[78,82] in fistulae of cryptoglandular origin and associated with Crohn's disease. In those trial, they found that the ASCs use is safe and effective. With the phase Ⅲ trial[83], they aimed to further investigate the effectiveness and safety of ASCs in the treatment of cryptoglandular complex anal fistulae. In this latter multicentre, randomized, single-blinded clinical trial, 200 adult patients from 19 centres were randomly assigned to receive either 20 million adipose-derived stem cells (group A, 64 patients), another 20 million stem cells plus a glue containing human fibrinogen and thrombin and bovine aprotin (group B, 60 patients) or fibrin glue (group C, 59 patients), all after closure of the internal opening. After 1 year of follow-up, they achieved a healing rate of 57%, 52% and 37% in group A, B and C respectively. No statistically significant differences were found when the 3 groups were compared. The use of ASCs was proved to be safe without any incontinence risk or procedure-related adverse events; however, its healing rate was not superior to fibrin glue alone and lower than other sphincter-preserving procedures.

To date, it is difficult to make a firm judgment of this technique because available data is limited. Many questions still remain unanswered, including whether the stem-cell suspension can be placed over the fistula tract and how much financially viable this technique is.

CONCLUSION

Currently, we confirm that the variety of procedures that can be used to treat fistulae, which testifies to the variable complexity of anal fistula and its unpredictable and non-reproducible results.

To improve the healing rates, patient selection is imperative, and control of sepsis, identification of secondary extensions and tracts of the fistula from the anal canal before any attempt of repair, is strongly suggested. The advancement flap still remains the gold standard, but LIFT may potentially play a fundamental role in the future. Furthermore, the use of biological material could be an alternative. Unfortunately, to date, it's technically difficult from the available literature, to determine any comparison between strategies, as patient demographics and disease vary considerably. Results are often not reported relatively to the specific aetiology or classification, and there is not any universally accepted method of evaluation of fistula healing during the follow-up period.

For all these reasons, the optimal procedure is the one tailored to the individual fistula.

REFERENCES

1   **Westerterp M**, Volkers NA, Poolman RW, van Tets WF. Anal fistulotomy between Skylla and Charybdis. *Colorectal Dis* 2003; **5**: 549-551 [PMID: 14617238]

2   **van der Hagen SJ**, Baeten CG, Soeters PB, van Gemert WG. Long-term outcome following mucosal advancement flap for high perianal fistulas and fistulotomy for low perianal fistulas: recurrent perianal fistulas: failure of treatment or recurrent patient disease? *Int J Colorectal Dis* 2006; **21**: 784-790 [PMID: 16538494]

3   **Whiteford MH**, Kilkenny J, Hyman N, Buie WD, Cohen J, Orsay C, Dunn G, Perry WB, Ellis CN, Rakinic J, Gregorcyk S, Shellito P, Nelson R, Tjandra JJ, Newstead G. Practice parameters for the treatment of perianal abscess and fistula-in-ano (revised). *Dis Colon Rectum* 2005; **48**: 1337-1342 [PMID: 15933794]

4   **Parks AG**, Stitz RW. The treatment of high fistula-in-ano. *Dis Colon Rectum* 1976; **19**: 487-499 [PMID: 964106]

5   **Mizrahi N**, Wexner SD, Zmora O, Da Silva G, Efron J, Weiss EG, Vernava AM, Nogueras JJ. Endorectal advancement flap: are there predictors of failure? *Dis Colon Rectum* 2002; **45**: 1616-1621 [PMID: 12473884]

6   **Kodner IJ**, Mazor A, Shemesh EI, Fry RD, Fleshman JW, Birnbaum EH. Endorectal advancement flap repair of rectovaginal and other complicated anorectal fistulas. *Surgery* 1993; **114**: 682-689; discussion 689-690 [PMID: 8211682]

7   **Ortíz H**, Marzo J. Endorectal flap advancement repair and fistulectomy for high trans-sphincteric and suprasphincteric fistulas. *Br J Surg* 2000; **87**: 1680-1683 [PMID: 11122184]

8   **Soltani A**, Kaiser AM. Endorectal advancement flap for cryptoglandular or Crohn's fistula-in-ano. *Dis Colon Rectum* 2010; **53**: 486-495 [PMID: 20305451 DOI: 10.1007/DCR.0b013e3181ce8b01]

9   **Dudukgian H**, Abcarian H. Why do we have so much trouble treating anal fistula? *World J Gastroenterol* 2011; **17**: 3292-3296 [PMID: 21876616 DOI: 10.3748/wjg.v17.i28.3292]

10   **Shawki S**, Wexner SD. Idiopathic fistula-in-ano. *World J Gastroenterol* 2011; **17**: 3277-3285 [PMID: 21876614 DOI: 10.3748/wjg.v17.i28.3277]

11   **McCourtney JS**, Finlay IG. Setons in the surgical management of fistula in ano. *Br J Surg* 1995; **82**: 448-452 [PMID: 7613885]

12   **Malouf AJ**, Buchanan GN, Carapeti EA, Rao S, Guy RJ, Westcott E, Thomson JP, Cohen CR. A prospective audit of fistula-in-ano at St. Mark's hospital. *Colorectal Dis* 2002; **4**: 13-19 [PMID: 12780648]

13   **Nwaejike N**, Gilliland R. Surgery for fistula-in-ano: an audit of practise of colorectal and general surgeons. *Colorectal Dis* 2007; **9**: 749-753 [PMID: 17854294]

14   **Eitan A**, Koliada M, Bickel A. The use of the loose seton technique as a definitive treatment for recurrent and persistent high trans-sphincteric anal fistulas: a long-term outcome. *J Gastrointest Surg* 2009; **13**: 1116-1119 [PMID: 19238493 DOI: 10.1007/s11605-009-0826-6]

15   **Kelly ME**, Heneghan HM, McDermott FD, Nason GJ, Freeman C, Martin ST, Winter DC. The role of loose seton in the management of anal fistula: a multicenter study of 200 patients. *Tech Coloproctol* 2014; **18**: 915-919 [PMID: 24989839 DOI: 10.1007/s10151-014-1186-0]

16   **Buchanan GN**, Owen HA, Torkington J, Lunniss PJ, Nicholls RJ, Cohen CR. Long-term outcome following loose-seton technique for external sphincter preservation in complex anal fistula. *Br J Surg* 2004; **91**: 476-480 [PMID: 15048751]

17   **Thomson JP**, Ross AH. Can the external anal sphincter be preserved in the treatment of trans-sphincteric fistula-in-ano? *Int J Colorectal Dis* 1989; **4**: 247-250 [PMID: 2614223]

18   **Hammond TM**, Knowles CH, Porrett T, Lunniss PJ. The Snug Seton: short and medium term results of slow fistulotomy for idiopathic anal fistulae. *Colorectal Dis* 2006; **8**: 328-337 [PMID: 16630239]

19   **Theerapol A**, So BY, Ngoi SS. Routine use of setons for the treatment of anal fistulae. *Singapore Med J* 2002; **43**: 305-307 [PMID: 12380728]

20   **Williams JG**, MacLeod CA, Rothenberger DA, Goldberg SM. Seton treatment of high anal fistulae. *Br J Surg* 1991; **78**: 1159-1161 [PMID: 1958973]

21   **Quah HM**, Tang CL, Eu KW, Chan SY, Samuel M. Meta-analysis of randomized clinical trials comparing drainage alone vs primary sphincter-cutting procedures for anorectal abscess-fistula. *Int J Colorectal Dis* 2006; **21**: 602-609 [PMID: 16317550]

22   **Tokunaga Y**, Sasaki H, Saito T. Clinical role of a modified seton technique for the treatment of trans-sphincteric and supra-sphincteric anal fistulas. *Surg Today* 2013; **43**: 245-248 [PMID: 22661282 DOI: 10.1007/s00595-012-0211-z]

23   **Rojanasakul A**, Pattanaarun J, Sahakitrungruang C, Tantiphlachiva K. Total anal sphincter saving technique for fistula-in-ano; the ligation of intersphincteric fistula tract. *J Med Assoc Thai* 2007; **90**: 581-586 [PMID: 17427539]

24   **Hong KD**, Kang S, Kalaskar S, Wexner SD. Ligation of intersphincteric fistula tract (LIFT) to treat anal fistula: systematic review and meta-analysis. *Tech Coloproctol* 2014; **18**: 685-691 [PMID: 24957361 DOI: 10.1007/s10151-014-1183-3]

25   **Alasari S**, Kim NK. Overview of anal fistula and systematic review of ligation of the intersphincteric fistula tract (LIFT). *Tech Coloproctol* 2014; **18**: 13-22 [PMID: 23893217 DOI: 10.1007/s10151-013-1050-7]

26   **Shanwani A**, Nor AM, Amri N. Ligation of the intersphincteric fistula tract (LIFT): a sphincter-saving technique for fistula-in-ano. *Dis Colon Rectum* 2010; **53**: 39-42 [PMID: 20010348 DOI: 10.1007/DCR.0b013e3181c160c4]

27   **Ellis CN**. Outcomes with the use of bioprosthetic grafts to reinforce the ligation of the intersphincteric fistula tract (BioLIFT procedure) for the management of complex anal fistulas. *Dis Colon Rectum* 2010; **53**: 1361-1364 [PMID: 20847616 DOI: 10.1007/DCR.0b013e3181ec4470]

28   **Bleier JI**, Moloo H, Goldberg SM. Ligation of the intersphincteric fistula tract: an effective new technique for complex fistulas. *Dis Colon Rectum* 2010; **53**: 43-46 [PMID: 20010349 DOI: 10.1007/DCR.0b013e3181bb869f]

29   **Ooi K**, Skinner I, Croxford M, Faragher I, McLaughlin S. Managing fistula-in-ano with ligation of the intersphincteric fistula tract procedure: the Western Hospital experience. *Colorectal Dis* 2012; **14**: 599-603 [PMID: 21831102 DOI: 10.1111/j.1463-1318.2011.02723.x]

30   **Tan KK**, Tan IJ, Lim FS, Koh DC, Tsang CB. The anatomy of failures following the ligation of intersphincteric tract technique for anal fistula: a review of 93 patients over 4 years. *Dis Colon Rectum* 2011; **54**: 1368-1372 [PMID: 21979180 DOI: 10.1097/DCR.0b013e31822bb55e]

31   **Steiner RW**, Omachi AS. A Bartter's-like syndrome from capreomycin, and a similar gentamicin tubulopathy. *Am J Kidney Dis* 1986; **7**: 245-249 [PMID: 2420173 DOI: 10.1177/1553350613508018]

32   **Aboulian A**, Kaji AH, Kumar RR. Early result of ligation of the intersphincteric fistula tract for fistula-in-ano. *Dis Colon Rectum* 2011; **54**: 289-292 [PMID: 21304298 DOI: 10.1007/DCR.0b013e318203495d]

33   **Mushaya C**, Bartlett L, Schulze B, Ho YH. Ligation of intersphincteric fistula tract compared with advancement flap for complex anorectal fistulas requiring initial seton drainage. *Am J Surg* 2012; **204**: 283-289 [PMID: 22609079 DOI: 10.1016/j.amjsurg.2011.10.025]

34   **Abcarian AM**, Estrada JJ, Park J, Corning C, Chaudhry V, Cintron J, Prasad L, Abcarian H. Ligation of intersphincteric fistula tract: early results of a pilot study. *Dis Colon Rectum* 2012; **55**: 778-782 [PMID: 22706130 DOI: 10.1097/DCR.0b013e318255ae8a]

35   **Lo O**, Wei R, Foo D, Law WL. Ligation of intersphincteric fistula tract procedure for the management of cryptoglandular anal fistula. *Surg Pract* 2012; **16**: 120-121

36   **van Onkelen RS**, Gosselink MP, Schouten WR. Is it possible to improve the outcome of transanal advancement flap repair for high transsphincteric fistulas by additional ligation of the intersphincteric fistula tract? *Dis Colon Rectum* 2012; **55**: 163-166 [PMID: 22228159 DOI: 10.1097/DCR.0b013e31823c0f74]

37   **Chen TA**, Liu KY, Yeh CY. High ligation of the fistula track by lateral approach: a modified sphincter-saving technique for advanced anal fistulas. *Colorectal Dis* 2012; **14**: e627-e630 [PMID: 22507907 DOI: 10.1111/j.1463-1318.2012.03050.x]

38   **Lehmann JP**, Graf W. Efficacy of LIFT for recurrent anal fistula. *Colorectal Dis* 2013; **15**: 592-595 [PMID: 23320466 DOI: 10.1111/codi.12104]

39   **Liu WY**, Aboulian A, Kaji AH, Kumar RR. Long-term results of ligation of intersphincteric fistula tract (LIFT) for fistula-in-ano. *Dis Colon Rectum* 2013; **56**: 343-347 [PMID: 23392149]

40   **Ellis CN**. Outcomes after repair of rectovaginal fistulas using bioprosthetics. *Dis Colon Rectum* 2008; **51**: 1084-1088 [PMID: 18478298 DOI: 10.1007/s10350-008-9339-8]

41   **Johnson EK**, Gaw JU, Armstrong DN. Efficacy of anal fistula plug vs. fibrin glue in closure of anorectal fistulas. *Dis Colon Rectum* 2006; **49**: 371-376 [PMID: 16421664]

42   **Ueno T**, Pickett LC, de la Fuente SG, Lawson DC, Pappas TN. Clinical application of porcine small intestinal submucosa in the management of infected or potentially contaminated abdominal defects. *J Gastrointest Surg* 2004; **8**: 109-112 [PMID: 14746842]

43   **Franklin ME**, Gonzalez JJ, Glass JL. Use of porcine small intestinal submucosa as a prosthetic device for laparoscopic repair of hernias in contaminated fields: 2-year follow-up. *Hernia* 2004; **8**: 186-189 [PMID: 14991410]

44   **Champagne BJ**, O'Connor LM, Ferguson M, Orangio GR, Schertzer ME, Armstrong DN. Efficacy of anal fistula plug in closure of cryptoglandular fistulas: long-term follow-up. *Dis Colon Rectum* 2006; **49**: 1817-1821 [PMID: 17082891]

45   **O'Connor L**, Champagne BJ, Ferguson MA, Orangio GR, Schertzer ME, Armstrong DN. Efficacy of anal fistula plug in closure of Crohn's anorectal fistulas. *Dis Colon Rectum* 2006; **49**: 1569-1573 [PMID: 16998638]

46   **van Koperen PJ**, D'Hoore A, Wolthuis AM, Bemelman WA, Slors JF. Anal fistula plug for closure of difficult anorectal fistula: a prospective study. *Dis Colon Rectum* 2007; **50**: 2168-2172 [PMID: 17701369]

47   **Ellis CN**. Bioprosthetic plugs for complex anal fistulas: an early experience. *J Surg Educ* 2007; **64**: 36-40 [PMID: 17320804]

48   **Lawes DA**, Efron JE, Abbas M, Heppell J, Young-Fadok TM. Early experience with the bioabsorbable anal fistula plug. *World J Surg* 2008; **32**: 1157-1159 [PMID: 18373120 DOI: 10.1007/s00268-008-9504-1]

49   **Ky AJ**, Sylla P, Steinhagen R, Steinhagen E, Khaitov S, Ly EK. Collagen fistula plug for the treatment of anal fistulas. *Dis Colon Rectum* 2008; **51**: 838-843 [PMID: 18330649 DOI: 10.1007/s10350-007-9191-2]

50   **Schwandner O**, Stadler F, Dietl O, Wirsching RP, Fuerst A. Initial experience on efficacy in closure of cryptoglandular and Crohn's transsphincteric fistulas by the use of the anal fistula plug. *Int J Colorectal Dis* 2008; **23**: 319-324 [PMID: 18038233]

51   **Garg P**. To determine the efficacy of anal fistula plug in the treatment of high fistula-in-ano: an initial experience. *Colorectal Dis* 2009; **11**: 588-591 [PMID: 18637937 DOI: 10.1111/j.1463-1318.2008.01632.x]

52   **Christoforidis D**, Etzioni DA, Goldberg SM, Madoff RD, Mellgren A. Treatment of complex anal fistulas with the collagen fistula plug. *Dis Colon Rectum* 2008; **51**: 1482-1487 [PMID: 18521674 DOI: 10.1007/s10350-008-9374-5]

53   **Thekkinkattil DK**, Botterill I, Ambrose NS, Lundby L, Sagar PM, Buntzen S, Finan PJ. Efficacy of the anal fistula plug in complex anorectal fistulae. *Colorectal Dis* 2009; **11**: 584-587 [PMID: 18637922 DOI: 10.1111/j.1463-1318.2008.01627.x]

54   **Safar B**, Jobanputra S, Sands D, Weiss EG, Nogueras JJ, Wexner SD. Anal fistula plug: initial experience and

outcomes. *Dis Colon Rectum* 2009; **52**: 248-252 [PMID: 19279419 DOI: 10.1007/DCR.0b013e31819c96ac]

55   **Ortiz H**, Marzo J, Ciga MA, Oteiza F, Armendáriz P, de Miguel M. Randomized clinical trial of anal fistula plug versus endorectal advancement flap for the treatment of high cryptoglandular fistula in ano. *Br J Surg* 2009; **96**: 608-612 [PMID: 19402190 DOI: 10.1002/bjs.6613]

56   **El-Gazzaz G**, Zutshi M, Hull T. A retrospective review of chronic anal fistulae treated by anal fistulae plug. *Colorectal Dis* 2010; **12**: 442-447 [PMID: 19220379 DOI: 10.1111/j.1463-1318.2009.01802.x]

57   **Chan S**, McCullough J, Schizas A, Vasas P, Engledow A, Windsor A, Williams A, Cohen CR. Initial experience of treating anal fistula with the Surgisis anal fistula plug. *Tech Coloproctol* 2012; **16**: 201-206 [PMID: 22527917 DOI: 10.1007/s10151-012-0810-0]

58   **Cintron JR**, Abcarian H, Chaudhry V, Singer M, Hunt S, Birnbaum E, Mutch MG, Fleshman J. Treatment of fistula-in-ano using a porcine small intestinal submucosa anal fistula plug. *Tech Coloproctol* 2013; **17**: 187-191 [PMID: 23053440 DOI: 10.1007/s10151-012-0897-3]

59   **Amrani S**, Zimmern A, O'Hara K, Corman ML. The Surgisis AFP anal fistula plug: a new and reasonable alternative for the treatment of anal fistula. *Gastroenterol Clin Biol* 2008; **32**: 946-948 [PMID: 18947949 DOI: 10.1016/j.gcb.2008.09.003]

60   The Surgisis AFP anal fistula plug: report of a consensus conference. *Colorectal Dis* 2008; **10**: 17-20 [PMID: 18028469]

61   **Buchberg B**, Masoomi H, Choi J, Bergman H, Mills S, Stamos MJ. A tale of two (anal fistula) plugs: is there a difference in short-term outcomes? *Am Surg* 2010; **76**: 1150-1153 [PMID: 21105632]

62   **de la Portilla F**, Rada R, Jiménez-Rodríguez R, Díaz-Pavón JM, Sánchez-Gil JM. Evaluation of a new synthetic plug in the treatment of anal fistulas: results of a pilot study. *Dis Colon Rectum* 2011; **54**: 1419-1422 [PMID: 21979188 DOI: 10.1097/DCR.0b013e31822c4d59]

63   **Ratto C**, Litta F, Parello A, Donisi L, Zaccone G, De Simone V. Gore Bio-A® Fistula Plug: a new sphincter-sparing procedure for complex anal fistula. *Colorectal Dis* 2012; **14**: e264-e269 [PMID: 22288601 DOI: 10.1111/j.1463-1318.2012.02964.x]

64   **Ommer A**, Herold A, Joos A, Schmidt C, Weyand G, Bussen D. Gore BioA Fistula Plug in the treatment of high anal fistulas--initial results from a German multicenter-study. *Ger Med Sci* 2012; **10**: Doc13 [PMID: 22984363 DOI: 10.3205/000164]

65   **Hjortrup A**, Moesgaard F, Kjaergård J. Fibrin adhesive in the treatment of perineal fistulas. *Dis Colon Rectum* 1991; **34**: 752-754 [PMID: 1914738]

66   **Buchanan GN**, Bartram CI, Phillips RK, Gould SW, Halligan S, Rockall TA, Sibbons P, Cohen RG. Efficacy of fibrin sealant in the management of complex anal fistula: a prospective trial. *Dis Colon Rectum* 2003; **46**: 1167-1174 [PMID: 12972959]

67   **Patrlj L**, Kocman B, Martinac M, Jadrijevic S, Sosa T, Sebecic B, Brkljacic B. Fibrin glue-antibiotic mixture in the treatment of anal fistulae: experience with 69 cases. *Dig Surg* 2000; **17**: 77-80 [PMID: 10720836]

68   **Swinscoe MT**, Ventakasubramaniam AK, Jayne DG. Fibrin glue for fistula-in-ano: the evidence reviewed. *Tech Coloproctol* 2005; **9**: 89-94 [PMID: 16007368]

69   **Sentovich SM**. Fibrin glue for anal fistulas: long-term results. *Dis Colon Rectum* 2003; **46**: 498-502 [PMID: 12682544]

70   **Cintron JR**, Park JJ, Orsay CP, Pearl RK, Nelson RL, Abcarian H. Repair of fistulas-in-ano using autologous fibrin tissue adhesive. *Dis Colon Rectum* 1999; **42**: 607-613 [PMID: 10344682]

71   **Venkatesh KS**, Ramanujam P. Fibrin glue application in the treatment of recurrent anorectal fistulas. *Dis Colon Rectum* 1999; **42**: 1136-1139 [PMID: 10496552]

72   **Lindsey I**, Smilgin-Humphreys MM, Cunningham C, Mortensen NJ, George BD. A randomized, controlled trial of fibrin glue vs. conventional treatment for anal fistula. *Dis Colon Rectum* 2002; **45**: 1608-1615 [PMID: 12473883]

73   **Wilhelm A**. A new technique for sphincter-preserving anal fistula repair using a novel radial emitting laser probe. *Tech Coloproctol* 2011; **15**: 445-449 [PMID: 21845480]

74   **Giamundo P**, Geraci M, Tibaldi L, Valente M. Closure of fistula-in-ano with laser--FiLaC™: an effective novel sphincter-saving procedure for complex disease. *Colorectal Dis* 2014; **16**: 110-115 [PMID: 24119103 DOI: 10.1111/codi.12440]

75   **Oztürk E**, Gülcü B. Laser ablation of fistula tract: a sphincter-preserving method for treating fistula-in-ano. *Dis Colon Rectum* 2014; **57**: 360-364 [PMID: 24509460 DOI: 10.1097/DCR.0000000000000067]

76   **Meinero P**, Mori L. Video-assisted anal fistula treatment (VAAFT): a novel sphincter-saving procedure for treating complex anal fistulas. *Tech Coloproctol* 2011; **15**: 417-422 [PMID: 22002535 DOI: 10.1007/s10151-011-0769-2]

77   **Garcia-Olmo D**, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005; **48**: 1416-1423 [PMID: 15933795]

78   **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 [PMID: 19273960 DOI: 10.1007/DCR.0b013e3181973487]

79   **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**: 211-228 [PMID: 11304456]

80   **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295 [PMID: 12475952]

81   **Aust L**, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy* 2004; **6**: 7-14 [PMID: 14985162]

82   **Guadalajara H**, Herreros D, De-La-Quintana P, Trebol J, Garcia-Arranz M, Garcia-Olmo D. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis* 2012; **27**: 595-600 [PMID: 22065114 DOI: 10.1007/s00384-011-1350-1]

83   **Herreros MD**, Garcia-Arranz M, Guadalajara H, De-La-Quintana P, Garcia-Olmo D. 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**: 762-772 [PMID: 22706128]