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ORIGINAL ARTICLE

Observational Study

Recurrent anal fistulae: Limited surgery supported by stem cells

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Author contributions: Garcia-Olmo D and Guadalajara H contributed equally to the design of the study, performed the surgical procedures, and acquired and analyzed all data, supervised the project, and wrote the first draft of the paper; Rubio-Perez I collaborated in the analysis and interpretation of data, critically revised the main text and content, and wrote the final version of the paper; Herreros MD collaborated in design and conception of the study and performed the surgical interventions; de-la-Quintana P contributed to the first acquisition of data from patients in the outpatient clinics and their analysis; Garcia-Arranz M provided cell resources and managed all regulatory and legal aspects related to the study, participating in design and conception, and contributed to the revision of contents related to cell behavior and physiology; all authors revised and approved the final version to be published.
Ethics approval: This work recruited patients under a Compassionate-use Program, which is legislated by the Royal Spanish Decree 1015/2009, 19th of July. We had to make an individual request for every patient to the Spanish Agency for Medicines and Health Products (AEMPS).
Informed consent: Consents were signed by the attending surgeons and the patients.
Conflict-of-interest: García-Olmo D and Garcia-Arranz M, have applied for two patents related with this study entitled "Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue" (WO 2006/057649) and "Use of adipose tissue-derived stromal stem cells in treating fistula" (WO 2006/136244). García-Olmo D is a member of the Advisory Board of Tigenix SAU. This manuscript has not been published nor has been presented as a podium/poster presentation in a scientific meeting.
Data sharing: Participants gave informed consent for sharing of patient data. The risk of identification is very low, as the research is committed to keeping the anonymity of the patients.

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Abstract

AIM: To study the results of stem-cell therapy under a Compassionate-use Program for patients with recurrent anal fistulae.

METHODS: Under controlled circumstances, and approved by European and Spanish laws, a Compassionate-use Program allows the use of stem-cell therapy for patients with very complex anal fistulae. Candidates had previously undergone multiple surgical interventions that had failed to resolve the fistulae, and presented symptomatic recurrence. The intervention consisted of limited surgery (with closure of the internal opening), followed by local implant of stem cells in the fistula-

tract wall. Autologous expanded adipose-derived stem cells were the main cell type selected for implant. The first evaluation was performed on the 8th postoperative week; outcome was classified as response or partial response. Evaluation one year after the intervention confirmed if complete healing of the fistula was achieved.

RESULTS: Ten patients (8 male) with highly recurrent and complex fistulae were treated (mean age: 49 years, range: 28-76 years). Seven cases were non-Crohn's fistulae, and three were Crohn's-associated fistulae. Previous surgical attempts ranged from 3 to 12. Two patients presented with preoperative incontinence (Wexner scores of 12 and 13 points). After the intervention, six patients showed clinical response on the 8th postoperative week, with a complete cessation of suppuration from the fistula. Three patients presented a partial response, with an evident decrease in suppuration. A year later, six patients (60%) remained healed, with complete reepithelization of the external opening. Postoperative Wexner Scores were 0 in six cases. The two patients with previous incontinence improved their scores from 12 to 8 points and from 13 to 5 points. No adverse reactions or complications related to stem-cell therapy were reported during the study period.

CONCLUSION: Stem cells are safe and useful for treating anal fistulae. Healing can be achieved in severe cases, sparing fecal incontinence risk, and improving previous scoring.

Key words: Adipose-derived stem cells; Cell therapy; Compassionate use; Crohn's disease; Fistula-in-ano

Core tip: Our group has performed various clinical trials with adipose stem cells. Patients with very complex fistulae, multiple previous surgeries, and treatment failure are generally not able to enter these studies despite the benefit and "last chance" of cure. We present the results of a Compassionate-use Program, which enabled the application of stem-cell therapy to these patients, under strict regulations. Ten patients were treated, and after one year of follow-up, we conclude that adipose stem cells are effective and safe, and 60% of the patients achieved complete healing.

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INTRODUCTION

A limited surgical treatment in recurrent perianal fistulae often results in new recurrence, whereas there is a high risk of fecal incontinence if an extensive surgical treatment is performed[1-3]. The use of stem cells to treat complex fistulae is a promising area of research[4,5], for they may help to regenerate damaged perianal tissue. Especially in Crohn's disease, the presence of these cells could favor healing through anti-inflammatory and immunomodulatory effects[6-9]. Various randomized controlled trials using stem cells for the treatment of anal fistulae have already been conducted, and all of them show an excellent safety profile. Nevertheless, the real efficacy is currently difficutl to assess[5]. A recent Spanish study revealed that the mean annual global cost of conventional treatments for patients with Crohn's disease and perianal fistulae is > € 8000/year[10].

According to current regulatory issues at the time (2002), our team started a clinical trial process in order to test the ability of adipose-derived stem cells (ASC) to improve healing in complex perianal fistulae, including those associated with Crohn's disease. The chosen cell source was adipose tissue because the harvesting process for ASC following liposuction was simple and could be performed in our on-site laboratory[11]. To the date, we have finished a complete clinical trial process: a pilot study[12], and Phase Ⅱ[13] and Phase Ⅲ clinical trials[14]. Although a complex perianal fistula is the worst scenario, we observed satisfactory healing in our patients, without associated fecal incontinence. We are currently developing novel clinical trials directed to test different strategies in order to improve our results[5].

Some of the patients with multi-recurrent anal fistulae did not meet the strict eligibility criteria of clinical trials or were scheduled in control groups. The only option to treat these fistulae with stem cells was by "compassionate use". To achieve this, the European regulatory laws and the Spanish Medicine Agency guidelines were followed in order to obtain regulatory permissions. Under the Compassionate-use Program, the surgical technique and the cells' lineage is tailored for each patient, reinforcing the possibilities of cure, as opposed to the clinical trial setting. In these special cases, we performed minimal surgical maneuvers (limited surgery) directed to the conditioning of the surgical field, followed by implant of cells, in order to improve healing. This strategy enabled us to avoid anal sphincter injury and facilitated cell homing[6].

The aim of this paper is to report our experience in a clinical trial- complementary Compassionate-use Program, and discuss the possible clinical uses of stem cells in the future, focusing on the treatment of complex and recurrent perianal fistulae.

Table 1 Study data

ID	Sex	Age (yr)	Crohn's disease	Park's classification	Previous surgical attempts	Initial incontinence score (Wexner)	Surgical technique	Fibrin glue	Cells	Response 8th week	Incontinence score 8th week	Healing one year after
1	Male	58	No	Ⅲ	8	1	Flap + deep curettage	No	eASC	Yes	0	Yes
2	Male	43	No	Ⅱ	4	Unknown	IO closure + partial fistulectomy	Yes	eASC	Yes	4	No
3	Male	76	No	Ⅲ	5	0	IO closure + deep curettage	Yes	eASC	Partial	0	No
4	Male	57	No	Ⅲ	12	0	IO closure + deep curettage	Yes	eASC	Yes	0	Yes
5	Female	45	Yes	Ⅳ (multiple tracts)	6	12	IO closure + deep curettage	Yes	eASC	Partial	8	Yes
6	Male	35	Yes	Ⅱ (stenosis)	5	Unknown	IO closure + deep curettage	No	eASC	No	0	No
7	Male	40	Yes	Ⅲ	3	Unknown	IO closure + deep curettage	Yes	eASC	Yes	0	Yes
8	Male	59	No	Ⅱ	11	13	Flap + deep curettage	No	eASC Allog	Partial	5	No
9	Male	50	No	I	3	Unknown	Fistulotomy	Yes	eASC	Yes	0	Yes
10	Female	28	No	Ⅲ	5 + ileostomy	Not evaluable	Flap + partial fistulectomy	Yes	SVF	Yes	Not evaluable	Yes

IO: Internal opening; eASC: Expanded adult stem cells; Allog: Allogeneic; SVF: Stromal vascular fraction.

MATERIALS AND METHODS

We present an observational study, including 10 patients (8 male and 2 female) with recurrent perianal fistulae who had previously undergone at least three surgical interventions (maximum: 12, average: 6.2), with failure to resolve the fistula. The mean age of the patients was 49 years, and ranged from 28 to 76 years (Table 1). Seven patients presented complex non-Crohn's fistulae (four were Parks' type Ⅲ)[15] and three patients had Crohn's-associated perianal fistulae. Two of these patients complained of fecal incontinence at the moment of enrollment in this study, with a Wexner Score[16] > 10.

Autologous expanded adipose-derived stem cells (eASC) were selected in eight cases. Another case was treated using stromal vascular fraction (SVF) and in the last one, allogeneic adipose derived stem cells (Allo-eASC) were employed.

Both eASC (autologous and allogeneic) and SVF protocols were approved by the Ethics Committee of La Paz University Hospital in accordance with Spanish law, and by the Spanish Medical Agency according to European Medicine Agency (EMA) guidelines. All patients signed a detailed informed consent prior to any intervention, which included permission for data publication. Our institutional Committee on Human Experimentation (La Paz University Hospital) supervised all interventions performed. All ethical standards were in accord with those of the Helsinki Declaration (1975).

SVF from lipoaspirate

The liposuction was performed by a plastic surgeon and obtained 80-100 mL of fat. Phosphate buffered saline (PBS; Gibco of Thermo Fisher Scientific, Waltham, MA, United States) was used to wash the raw lipoaspirate and remove local anesthetics and cells. To extract the cellular fraction, the washed fat was digested with type Ⅰ collagenase (Gibco) at a final concentration of 0.075% in saline solution at 37 ℃ for 45 min.

Collagenase was inactivated with Dulbecco's modified Eagle's medium (DMEM; Gibco) containing fetal bovine serum (10% v/v). Cells in suspension were then centrifuged for 10 min (250 × *g*) and PBS was used again to wash the pellet. Centrifugation was repeated and afterwards the remaining erythrocytes were lysed by treating the suspension with ammonium chloride 160 mmol/L for 10 min at room temperature. To conclude the cellular extraction, a final wash and a filtration of the product through a 40 μm nylon mesh was performed.

Before injection, cells were suspended in sterile ringer-lactate solution (Griffols S.A., Barcelona, Spain). Morphologic determinations and phenotypic analyses were performed during product obtention. Data are partly published in García-Olmo *et al*[12]. The cell viability was always > 95% as determined by trypan-blue (Sigma-Aldrich, St Louis, MO, United States).

Autologous stem cell expansion and preparation for implantation

The released cellular fraction (SVF) was seeded at 2-3 × 10^4 cells/cm^2. Culture was carried out in DMEM with 10% fetal bovine serum and 1% ampicillin/streptomycin. No additional supplements were added. The atmospheric conditions were 37 ℃ with a 5% CO_2 atmosphere.

Cells were re-plated once 80% confluency was confirmed; their prior detachment was performed by trypsinization (trypsin-EDTA; Gibco). This cycle was repeated up to three times until the required number of cells for implantation was obtained. Due to logistics and personal issues, in two cases the cells were then frozen for preservation.

Morphologic determinations and phenotypic analyses were performed by flow cytometry during

expansion. Mycoplasma was detected using a Myco Alert Mycoplasma Detection Kit (Cambrex Corp., East Rutherford, NJ, United States). Data are partly published in García-Olmo *et al*[12].

At least one week before the surgical intervention was scheduled, expanded ASCs were prepared (washed with PBS, trypsinized, and centrifuged). Their viability was checked (> 95%), and finally the cells were resuspended in ringer-lactate solution (Griffols S.A.) at the desired volume and concentration (depending on the fistula) for their immediate use.

Allogeneic stem cell expansion and preparation for implantation

These cells were manufactured from donors by Tigenix SAU (Madrid, Spain) according to EMA permissions and regulations from healthy donors. The expansion protocol was similar to that of autologous procedures.

Treatment procedure and evaluation of healing

All surgical procedures were performed at La Paz University Hospital (Madrid), by the same team of surgeons, belonging to the Colorectal Surgery Unit.

In all cases, a deep curettage of the tracts was first performed, and then the ASC suspension (50%) was injected through a long, fine needle into the tract walls. The injections were superficial; not deeper than 2 mm. In seven cases, the fistulous tract was sealed with fibrin glue (Baxter Inc., Deerfield, IL, United States) containing a portion (1 mL) of the cells. The fibrin glue was used as a sealant to finalize the procedure in order to ensure cells remained in the fistulous area. The main reason for injecting a percentage of the ASC into the fibrin glue was to have a reservoir in the area so they could act for longer. However, recent investigations indicate that cells alone are sufficient for a therapeutic effect[17,18].

In very complex perianal fistulae, a partial fistulectomy was performed without removing intra-sphincteric tracts. The closure of the internal opening was achieved by stitches in six cases and by a mucosal advancement-flap in three cases. In the remaining patient, a fistulotomy was performed.

Treatment outcomes

A first evaluation was performed on the 8th post-toperative week, and a final evaluation was scheduled one year after the procedure (although patients attended the outpatient clinic in between, at variable intervals). Response was defined as a complete cessation of suppuration on week 8, despite not achieving a complete re-epithelization. Partial response was defined as an evident decrease in suppuration. Healing was defined as no suppuration from the external orifice, achieving a complete re-epithelization after one year of follow-up. These intervals of time for follow-up were selected following published data about the best periods for long-term fistula follow-up evaluation[19].

RESULTS

Of the ten highly recurrent perianal fistulae treated, 6/10 (60%) showed a clinical response 8 wk after the procedure, and 3/10 (30%) showed a partial response. One year later, 6/10 (60%) remained healed, with the external opening being completely epithelialized (Table 1). Postoperative results of Wexner Scores for Incontinence[16] were 0 in 6 cases. In the two patients with previous fecal incontinence, the scoring improved from 12 to 8 and from 13 to 5. No adverse reactions or complications related to stem-cell therapy were reported during the study period.

No statistical relationships have been established between the use of fibrin glue, surgical approach or cell lineage, due to the small number and variability of patients.

DISCUSSION

Following strict regulations, we treated ten patients with recurrent perianal fistulae, achieving a 90% response and a complete healing after one year in six cases, with no associated incontinence. Moreover, in two cases, previous incontinence was reduced. It is important to remark that the performance of this therapeutic strategy does not produce injury to the anal sphincter, because intrasphincteric-tract resection is not required. Although this is not a randomized controlled trial, the results are similar to those already published[12-14].

ASCs enlarge the therapeutic arsenal for anal fistulae, and can be considered an interesting tool for the regeneration/repair of wounds or chronically damaged tissues. The specific mechanism of action of ASCs is still under study, but it has been widely demonstrated that these cells improve healing[6]. Two different biologic effects are responsible for this healing effect: proliferation and differentiation on the one hand, and immune regulation and local suppression of inflammation on the other[6].

According to the EMA, ASC treatments in the EU should be administered only under clinical trials or other controlled conditions, such as Compassionate-use Programs. It is important to remark that all clinical uses of stem cells outside of these regulatory conditions are considered illegal. This is clearly stated in the law RD 1015/2009. In this way, in February 2011, the EMA published a report on stem cell-based medicinal products. It expressed concerns about the unregulated use of medicinal products containing ASCs.

In this context, one of the limitations of our study is the small number of patients included. We believed that a limited surgical treatment supported by ASC could be beneficial for these patients; but only those that did not meet the inclusion criteria or were

Table 2 Published studies on stem cell therapy for anal fistulae

Ref.	Year	Condition	Study design	Cell source	Cell quantity (dose)	Intervention model
García-Olmo *et al*[11]	2003	Recto-vaginal fistula in Crohn's disease	Case report	Autologous eASC	1×10^7	Single arm
García-Olmo *et al*[12]	2005	Enterocutaneous, recto-vaginal, perianal fistula in Crohn's disease	Phase Ⅰ	Autologous eASC	1-3 × 10^7 re-suspended in fibrin glue	Single arm
García-Olmo *et al*[13]	2009	Perianal fistula with or without Crohn's disease	Phase Ⅱ	Autologous eASC	Not specified	Two arms: fibrin glue, fibrin glue + eASC
García-Olmo *et al*[22]	2010	Recto-vaginal fistula in Crohn's disease	Case report	Allogenic eASC	Not specified	Single arm
Ciccocioppo *et al*[25]	2011	Enterocutaneous and complex perianal fistula in Crohn's disease	Case report	Expanded autologous bone marrow	5×10^7	Single arm
Cho *et al*[24]	2012	Perianal fistula in Crohn's disease	Phase Ⅰ	Autologous eASC	Not specified	Single arm: dose escalation study
Herreros *et al*[14]	2012	Complex perianal fistula without Crohn's disease	Phase Ⅲ	Autologous eASC	2×10^7 then 4×10^7 if no effect	Three arms: fibrin glue, eASC, fibrin glue + eASC
Herreros *et al*[14]	2012	Complex perianal fistula without Crohn's disease	Observational	Autologous eASC	2×10^7 then 4×10^7 if no effect	Three arms: fibrin glue, eASC, fibrin glue + eASC
Guadalajara *et al*[23]	2012	Perianal fistula with or without Crohn's disease	Observational	Autologous eASC	Not specified	Two arms: fibrin glue, fibrin glue + eASC
de la Portilla *et al*[17]	2012	Perianal fistula in Crohn's disease	Phase Ⅰ / Ⅱ	Allogeneic eASC	2×10^7 then 4×10^7 if no effect	Single arm
Lee *et al*[20]	2013	Perianal fistula in Crohn's disease	Phase Ⅱ	Autologous eASC	Depending on the fistula. Re-dosing (1.5 times) if no effect	Single arm

SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: Expanded adult stem cells.

scheduled in control groups of our clinical trials could be selected for the present study.

Various randomized controlled trials using ASCs for the treatment of anal fistulae have been conducted, and all of them show an excellent safety profile. Nevertheless, the actual efficacy is difficult to assess. To date, there are 11 published papers including data on stem cell-based treatment of anal fistulae (Table 2). The first one was published in 2003[11], and the last was published very recently, in 2013[20]. Eight of these have been published by Spanish groups[11-14,17,21-23], two other papers come from South Korea[20,24], and one from Italy[25]. The majority refer to ASC treatment of Crohn's disease-related fistulae. The Italian study was the only one to select bone marrow as the ASC source for the treated fistula[25]. The rest of the studies employed autologous or allogeneic cells from adipose tissue. In all studies, cells were expanded, and a wide range of doses applied. Except for the Italian study[25], all procedures included closure of the internal opening, and in all cases the cell injections were intra-lesional. Over three hundred patients have been enrolled in these studies and the most important result is the assurance of the excellent safety profile of stem cells: no serious cell-related adverse events were described. Regarding efficacy, results show very different profiles, but about 40%-60% of patients achieved healing (Table 3).

The cost of the treatment is another important issue. Nowadays, the production cost of expanded ASCs (Good Manufacturing Practice compliant) can range from € 8000-12000, though the production could be reduced to € 3000-4000 without expansion[13]. We estimate that a high scale industrial production could significantly reduce the expenses. A reasonable strategy that we propose, considering the high cost of cell expansion and our previous results, would be to apply a first treatment with SVF, freeze a portion of the cells obtained, and propose a second treatment with expanded cells in cases achieving no response after eight weeks.

Treatment of recurrent fistulae is a difficult surgical challenge. In these patients, the pursuit of healing usually involves multiple operations, with a subsequent perianal scarring and distortion. In the most complex cases, the condition is worsened by the accompanying fecal incontinence. Therefore, these individuals are progressively more difficult to treat, resulting in the exasperation of both the patient and the surgeon[26]. In these cases, wound healing is a critical issue, and indeed, new approaches are needed. For the reasons outlined earlier, we believe that once available, ASCs will fulfill a clear and previously unmet medical need, helping to improve the healing and hence the quality of life of patients with recurrent perianal fistulae.

In conclusion, limited surgery supported by ASCs may constitute as a new therapeutic strategy in the treatment of recurrent fistulae.

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Table 3 Outcomes of published clinical experience of stem cell treatment for anal fistula

Ref.	Procedure	No. of patients treated	Healed (*n*)	Follow-up (mo)	Recurrence (*n*)	SAE (*n*)
García-Olmo *et al*[11]	Closure of IO. Injection in site, without fibrin glue	1	1	3	0	0
García-Olmo *et al*[12]	Cells resuspended in fibrin glue. Injection in site	9	6	12	Not specified	0
García-Olmo *et al*[13]	Closure of IO. Injection in site	Fibrin glue: 25 Fibrin glue + eASC: 24	Fibrin glue: 3 Fibrin glue + eASC: 17	12	Fibrin glue: 0 Fibrin glue + eASC: 2	4 (1 related to fibrin glue, others unrelated)
García-Olmo *et al*[22]	Closure of IO. Injection in site, without fibrin glue	1	1	36	1	0
Ciccocioppo *et al*[25]	Four injections in site	10	7	12	0	0
Cho *et al*[24]	Closure of IO and fibrin glue. Injection in site	9	3	15	0	0
Herreros *et al*[14]	Closure of IO. Injection in site	eASC: 64 Fibrin glue + eASC: 60 Fibrin glue: 59	eASC: 27 Fibrin glue + eASC: 24 Fibrin glue: 23	6	eASC: 0 Fibrin glue + eASC: 4 Fibrin glue: 0	4 unrelated to study treatment
Herreros *et al*[14]	Closure of IO. Injection in site	Not specified	eASC: 57% Fibrin glue+ eASC: 52.4% Fibrin glue: 37.3%	12	Not specified	1 unrelated to study treatment
Guadalajara *et al*[23]	Closure of IO. Injection in site	Fibrin glue: 13 Fibrin glue + eASC: 21	Fibrin glue: 3 Fibrin glue + eASC: 10	38	Fibrin glue: 1 Fibrin glue + eASC: 5	0
de la Portilla *et al*[17]	Closure of IO. Injection in site, without fibrin glue	24	9	4	Not specified	2 unrelated to study treatment
Lee *et al*[20]	Injection in site and fibrin glue	43	27	12	4	0

IO: Internal opening; SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: Expanded adult stem cells.

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COMMENTS

Background

The concept of stem cell therapy came from the possibility of obtaining an immature cell that could differentiate into a specific lineage if placed in the correct environment. One of the first examples was the transplantation of stem cells in hematologic patients, which could achieve the regeneration of normal marrow, and is now widely used. Following this idea, researchers raised the question of whether or not there could be different stem cells for other organs, or even embryonic cells that could develop into any of them. The study and therapeutic use of these types of cells could be the answer for many incurable injuries and diseases.

Research frontiers

Complex and recurrent anal fistulae (whether associated or not to Crohn's disease), constitute an important surgical problem, which can be difficult to solve. Many strategies have been proposed to achieve healing, including different surgical techniques, including fibrin glues and plugs. The application of stem cells in the fistula tract promotes the "closure" of the fistula by stimulating the regeneration of the tissue, both by direct growth and immunomodulatory effects. The exact mechanisms by which stem cells induce healing are still under investigation.

Innovations and breakthroughs

An increasing number of randomized controlled trials have tested the application of adipose-derived stem cells (ASCs) in perianal fistulae, with variable rates of success. In the present study, we applied ASCs to the worst patients, those with recurrent fistulae despite multiple previous treatments and interventions. In these desperate cases, even a partial response to the treatment was a success, as patients' distress was a constant after so many failures. ACSs were remarkably effective and we achieved healing in 60% after one year of follow-up.

Applications

This study, and various others in a randomized controlled trial setting, suggests that the surgical application of stem cells in anal fistula tract is a potentially therapeutic strategy that could resolve even the most recurrent and complex fistulae. In the same direction, ACSs are being used for the regeneration of skin, cartilage, bone, cornea, endothelium, *etc*. Applications to other organs, such as the heart and lung, and the nervous system have raised high expectations in the field. Research continues, and new applications are sure to develop in the near future.

Terminology

An anal fistula is an abnormal conduct communicating the anal canal with the perianal skin. The fistula tract typically breaks through the sphincters, and can have multiple ramifications. If one of the openings is blocked, an abscess occurs, which can worsen the condition. Drainage of these abscesses and surgical attempts to close the fistula can damage the muscle of the sphincters and cause fecal incontinence. The former, associated to suppuration and pain are the most common symptoms, creating a permanent discomfort for patients. Adult (somatic) stem cells are undifferentiated cells that can be found in differentiated tissue (such as bone, fat, and muscle) and have the potential to give rise to the specialized cell types present in the tissue from which they originate. A stem cell fulfills three characteristics: self-renewal capacity, differentiation potential, and *in vivo* engraftment capacity. Stem cell therapy: use of stem cells to replace those from damaged or diseased tissue. The source of the cells can either be the patient (autologous), another individual (allogeneic), or an animal (xenogeneic).

Peer-review

A good and interesting study even though it includes only ten patients. However, the results are very useful to speculate about the best current treatment of recurrent complex fistulae. It will be interesting if a randomized cross-over multicenter study can confirm these results with stem cell therapy in the complex anal fistulae.

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