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Prospect of Cell Therapy for Treating Perianal Fistula, Including Crohn's Disease

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Abstract

Introduction: Complex perianal fistula continues to be a real surgical challenge due to the high rate of recurrence and the possibility of fecal incontinence after surgery. In the case of treatment of fistulas in patients with Crohn's disease, the results produced to date have been disappointing in spite of the administration of biological drugs.

Areas Covered: Novel methods are needed for this condition, and cells appear to have potential to improve fistula healing. Mesenchymal stromal cells (MSCs) have been used in several clinical trials, including phase-III studies. After an analysis of the published papers we can conclude that MSCs have evident anti-inflammatory and immune-regulatory properties, MSCs are safe for clinical practice and have decent results considering the challenging conditions surrounding the procedure and the safety profile of the intervention. These trials can also raise important practical issues regarding cell source, dose levels, and method of implantation. The results of ongoing large clinical trials are pending.

Conclusion: According to current data, we can say that cell therapy may become a clinical reality for the treatment of complex perianal fistula and currently can be used as a support for the surgical maneuvers during perianal fistula surgery.

Keywords

Perianal fistula, Crohn's disease, Cell therapy, Outcomes review

Introduction

The incidence of perianal fistula among patients with Crohn's disease (CD) is 28% [1], while in patients without CD, only 2 cases per 10 000 people are detected each year [2]. Recurrent perianal fistulas pose a notoriously difficult surgical challenge and very often surgical attempts fails in these kind of patients. Even simple fistulas are difficult to treat, because the surgeon must access the anal sphincter, which may compromise the functioning of this structure and lead to fecal incontinence. In a cohort of 537 patients with low perianal fistula of cryptoglandular etiology treated by fistulotomy, major incontinence was reported in 28% at 5 years of follow-up [3].

Mucosal or full-thickness advancement flaps and other surgical techniques are less advantageous because post-surgical healing must take place in a septic and inflamed environment. This situation becomes more and more complicated with successive surgical attempts, causing further perianal scarring and distortion of the perianal area and making it difficult to correctly identify possible areas of sepsis. The inevitable result is that these individuals are progressively more difficult to treat, making both patient and surgeon more and more frustrated. Moreover, continuous suppuration in the anal region in unhealed or recurrent fistulas leaves the patient at risk of acute infection with abscess formation, requiring urgent surgical drainage.

Limited surgical treatment for perianal fistula in the context of CD often results in high rates of recurrence, whereas extensive surgical treatment may cause fecal incontinence. Treatments other than surgery are far from ideal due to high recurrence rates and morbidity. Biologic agents such as infliximab do not generally reach the goal of treatment [4-6].

The aim of this review is to discuss the use of cell therapy to treat complex perianal fistula in patients with or without CD, approaching this issue from a practical and surgical point of view.

The Rationale: Why Cells for Perianal Fistula Treatment?

Perianal fistula is very difficult to treat, and even small fistula tracts can be challenging to heal [3,7] due to the impairment of the healing process caused by this condition. In healthy patients, the healing processes is well known, and always consists of the following phases in the same order regardless of the tissue: i) inflammatory phase, ii) proliferative, regenerative, or reparative phase, and iii) maturational phase.

The inflammatory phase is perpetuated due to fecal impaction in the tract, constant contact with bacterial antigens, and infection, thus leading to the release of more pro-inflammatory mediators and

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subsequent deep tissue destruction and perpetuation of the cycle. This inflammatory phase is even more aggressive in CD [8,9]. During phase 2, myofibroblasts play an important role in coordinating the process [10]. Healing processes that are not well orchestrated may lead to epithelization of the deepest part of the wound [11]. The epithelium acts as a barrier, though in this case the barrier prevents the fistula tract from healing [12,13]. Consolidation of the scar occurs in the third phase, though in this area it may cause fibrosis of the anal sphincter [14], deformation of anal shape leading to incontinence, and further epithelization of tracts leading to perpetuation of the fistula [12,13].

This situation of fistula perpetuation is exacerbated in the case of CD due to an uncontrolled inflammatory response. Improper response to intraluminal intestinal flora causes disproportionate activation of CD4 lymphocytes and the release of a huge amount of cytokines, both of which are potentiated by the loss of apoptosis of these CD4 lymphocytes and the elevation of intestinal permeation [8]. In CD, we have also seen that the pattern of cells seen in the perianal fistula tracks are different from those of patients without CD who develop perianal fistula; in particular, fistulas in CD patients reveal myofibroblasts scattered along a fragmented underlying basement membrane, while non-CD fistulas have a well-ordered distribution, fewer CD68-positive macrophages, and more CD45RO-positive T cells [11]. These changes increase the difficulty of controlling the inflammation and healing processes.

In the scenario of perianal fistula, the use of cells has emerged as a novel means of improving wound healing. Cell transplantation provides a way of increasing the number of cells locally in these critical phases with the aim of restoring normal wound healing. In addition, a special kind of cells named mesenchymal stromal cells (MSCs) have become a potent partner in wound healing. MSCs were initially described as a population of bone-marrow-derived mononuclear cells with a fibroblast-like morphology. They later have been found in a wide range of tissues, such as fat. The immunomodulatory activities of MSCs described to date include: a) suppression of naive and memory CD4+; b) suppression of CD8+ T-cell proliferation and differentiation; c) promotion of regulatory T-cell expansion and enhancement of their immunosuppressive activity; d) impairment of dendritic cells; f) and secretion of nitric oxide, prostaglandin E2, hepatocyte growth factor, and indoleamine 2,3-dioxygenase [15]; g) Finally, MSCs have the ability to migrate to the site of injury, directing these immunomodulatory and regenerative properties to the exact place where they are needed [16].

Practical Issues in the Use of Cells to Treat Perianal Fistula

Sources of cells

MSCs have been obtained from numerous sources (e.g., placenta, [17] umbilical cord) [18] though in clinical settings, those obtained from bone marrow and fat have been used to treat perianal fistulas.

MSCs derived from bone marrow and fat have certain shared characteristics, such as plastic adherence, marker expression, and differentiation properties. Some differences between the 2 sources, however, make adipose-derived MSCs more advantageous because of the following reasons: a) more studies using fat have been published (13 clinical trials conducted with adipose-derived stromal cells, compared to only 3 with bone marrow); b) the number of MSCs in fat is 500 fold higher than in the bone marrow [15]; c) cells are easily isolated in raw form, making it possible to obtain enough cells for a treatment on the same day using liposuction (Figure 1), while the volume of cells obtained from bone marrow aspiration is lower and expansion of the cells is needed before treatment can be performed; d) in other clinical settings (osteonecrosis), adipose-derived stromal cells are phenotypically superior for regeneration [19]; e) ASCs show greater angiogenic potential [20]; f) compared to umbilical cord and adipose tissue-derived stromal cells, bone marrow MSCs had the lowest proliferative capacity [21]; g) and finally ASCs in long term culture are more stable genetically and morphologically [22]. On the other hand ASCs may not produce the expected result due to contamination by other cells types, especially fibroblast [23].

Routes of cell administration to treat perianal fistula

Two routes of administration have been tried: intravenous (systemic) and intralesional. The clinical trial NCT NCT00482092 (data

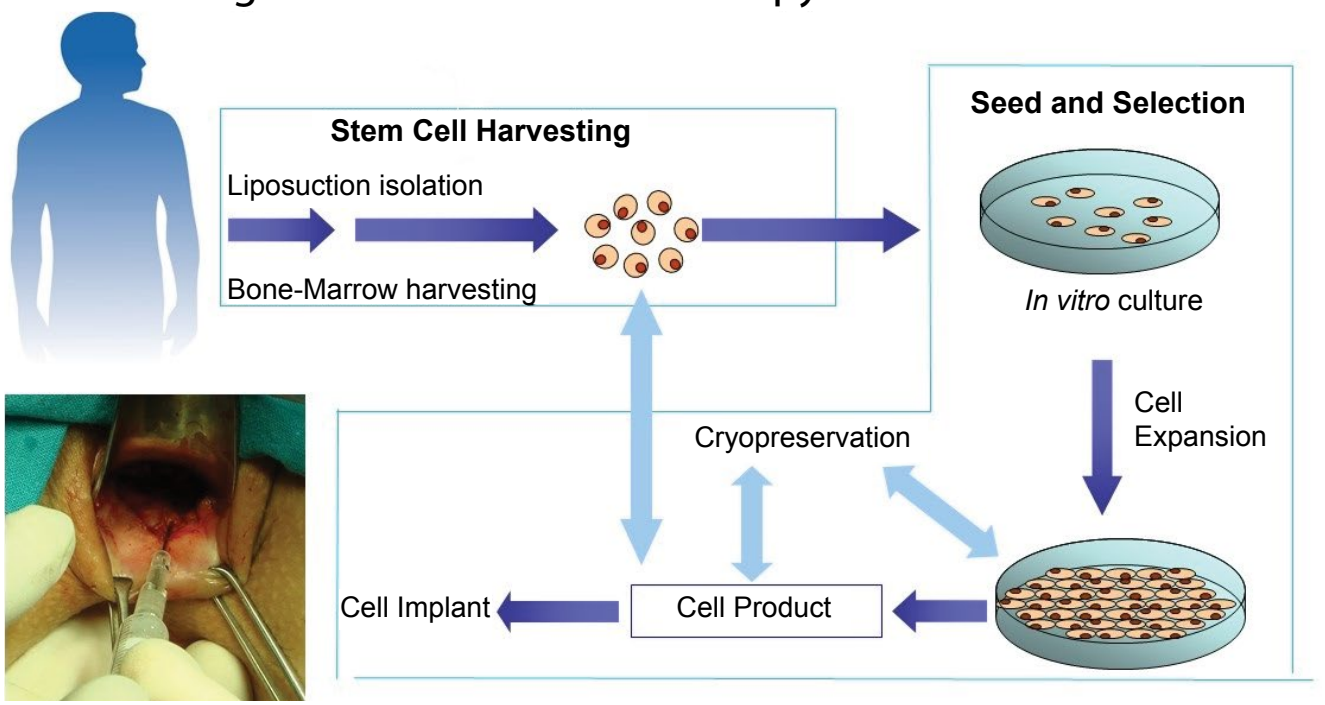

Figure 1: Technologies involved in cell therapy for perianal fistulas. Cells can be harvested by isolation from a product of liposuction or by harvesting from bone-marrow aspiration. This product can be injected in raw form after little manipulation. Later, these cells can be seeded in order to select specific MSCs based on their plastic adherent properties. Using "*in vitro*" culture technologies, we can expand cells until they achieve the adequate quantity (millions). The final cell product can be cryopreserved or administered directly. Moreover, from a cryopreserved product we can culture new doses for patient injection.

not published) is currently studying intravenous injection of allogeneic MSCs from bone marrow in patients with CD is being studied; however, treatment of the perianal fistula is a secondary endpoint.

To our knowledge, all other studies have been designed to deliver the cells inside the fistula track.

The search for suitable doses

The ideal dose of cells to treat fistula has yet to be determined. The dose used in published studies ranges from 1 × 10e7 to 9 × 10e7. A higher dose does not ensure better results. Moreover, the latest publication by Molendijk *et al.* has revealed that the group with the higher dose exhibits the worst results [24]; nonetheless, some of the trials currently in the recruitment phase or ongoing are using even higher numbers of cells.

Use of cells mixed with scaffolds

Many different scaffolds have been used in an attempt to regenerate tissues, and perianal fistula is one examples. Some studies have already been published using fibrin glue as a scaffold for the treatment of perianal fistula [25,26]. As published elsewhere, our experience is better when cells are combined with fibrin glue [27], and this observation has been confirmed by researchers from other fields such as orthopedic surgery [28].

Table 1: Published clinical experiences of cell treatments of anal fistula (Part 1). An update of the tables taken from the section "Stem Cell Application in Fistula Disease" [33].

Investigators	Year of publication	Trial code	Location	Condition	Study design	Cell source	Expanded	Cells number
García-Olmo *et al.* [34]	2003	NA	Spain	Recto-vaginal fistula in Crohn's disease	Case report	Autologous fat	Yes	1 × 10e7
García-Olmo *et al.* [41]	2005	Not registered	Spain	Enterocutaneous, recto-vaginal, perianal fistula in Crohn's disease	Phase I	Autologous fat	Yes	1-3 × 10e7 resuspended in fibrin glue
García-Olmo *et al.* [25]	2009	NCT00115466	Spain	Perianal fistula with or without Crohn's disease	Phase II	Autologous fat	Yes	Not specified
García-Olmo *et al.* [40]	2010	NA	Spain	Recto-vaginal fistula in Crohn's disease	Case report	Allogeneic fat	Yes	Not specified
Ciccocioppo *et al.* [39]	2011	NA	Italy	Enterocutaneous, complex perianal fistula in Crohn's disease	Case report	Autologous bone marrow	Yes	5 × 10e7
Herreros *et al.* [27]	2012	NCT00475410	Spain	Complex perianal fistula without Crohn's disease	Phase III	Autologous fat	Yes	2 × 10e7 then 4 × 10e7 when no effect
Herreros *et al.* [27]	2012	NCT01020825	Spain	Perianal fistula with or without Crohn's disease	Observational	Autologous fat	Yes	2 × 10e7 then 4 × 10e7 when no effect
Guadalajara *et al.* [42]	2012	Not registered	Spain	Perianal fistula with or without Crohn's disease	Observational	Autologous fat	Yes	Not specified
Borowski *et al.* [35]	2012	NA	UK	Perianal fistula without Crohn's disease	Case report	Autologous fat	No	Not specified
de la Portilla *et al.* [37]	2013	NCT01372969	Spain	Perianal fistula in Crohn's disease	Phase I/II	Allogeneic fat	Yes	2 × 10e7 then 4 × 10e7 when no effect
Cho *et al.* [38]	2013	NCT00992485	Korea	Complex perianal fistula in Crohn's disease	Phase I	Autologous fat	Yes	1 × 10e7, 2 × 10e7, 4 × 10e7
Lee *et al.* [36]	2013	NCT01011244	Korea	Complex perianal fistula in Crohn's disease	Phase II	Autologous fat	Yes	3 × 10e7 per cm length of the fistula. Initial dose × 1.5 when no effect
Cho *et al.* [43]	2015	NCT01011244 long term	Korea	Complex perianal fistula in Crohn's disease	Phase II	Autologous fat	Yes	3 × 10e7 per cm length of the fistula. Initial dose × 1.5 when no effect
Ciccocioppo *et al.* [44]	2015	NA	Italy	Enterocutaneous, complex perianal fistula in Crohn's disease	Case report	Autologous bone marrow	Yes	5 × 10e7
García-Olmo *et al.* [45]	2015	NA	Spain	Complex perianal fistula with or without Crohn's disease	Case report	Autologous fat	Both	Not specified
Molendijk *et al.* [24]	2015	NCT01144962	Netherlands	Perianal fistula in Crohn's disease	Phase I/II	Allogeneic bone marrow	Yes	1 × 10e7, 3 × 10e7, 9 × 10e7
Borowski *et al.* [46]	2015	NA	UK	Perianal fistula without Crohn's disease	Case report	Autologous fat	No	Not specified
Park KJ. [47]	2015	NCT01440699	Korea	Perianal fistula in Crohn's disease	Phase I	Allogeneic fat	Yes	1 × 10e7, 3 × 10e7

These improved results may be explained because in large fistula tracks and big cavities, cells are better distributed all along the area. It is true, however, that fistula track are sometimes very small, making straight injection more effective in terms of cell distribution.

Is important to remark that in our experience cells must be suspended in the fibrin component because of the potential for toxicity posed by thrombin.

Finally, a clinical trial has recently been registered on the siteclinicaltrials.gov (NCT01915927) to determine the safety and toxicity of using autologous MSC-coated fistula plug in patients with fistulizing CD.

Safety profile of MSCs

The biosafety of MSCs has been tested by several studies. To date we can assume at least the following: a) no oncological events reported in long-term studies, even in patients with long course of perianal CD, which in itself confers higher risk of cancer [29]; b) genetic stability studies have been performed, expanding MSCs from the bone marrow of sarcoma patients, without revealing any evidence of malignant transformation [30,31]; c) Studies of the newborns of patients treated with cells did not show any influence on fertility, course of pregnancy, newborn weight, or physical condition of newborns [32].

Regulatory affairs

In Europe, cells, which are regulated by the European Medicines Agency, can only be used in 2 scenarios: approved clinical trials or compassionate use (Regulation (EC) No 1394/2009 and Directive 2001/83/EC). In the United States, this issue is regulated by the Evaluations and Research division of the FDA, which has only approved minimally manipulated cell products for homologous use, although this situation is about to change toward another status that is similar to that of the European Union.

Review of Published Clinical Data

Despite the lack of results derived from long-term outcomes of cell therapy in perianal fistula, and the challenges of regulatory affairs adding more difficulties to the trials with cells, since our last review of this issue in 2014 [33], 5 new trials have been registered and 6 new studies have been published.

Published papers

The first publications to come out of cell-based trials for fistula treatment came from a single publication in 2003 and appeared as a proof-of-concept paper concerning a patient with recto-vaginal fistula and CD [34]. The treatment was successful, and since then many trials have been published, most of which have been open-label studies performed on a small number of patients. Nonetheless, the good results obtained are encouraging other researchers to explore this medical condition in larger trials, as seen in Table 1 and Table 2 [25,34-41]. The first multicenter phase-III trial, with an enrollment of 214 patients, was published in 2012 [27]. Although results were not entirely bad with a healing rate of 40% in complex fistulas with minimal intervention the results were similar between patients treated with fibrin glue and the experimental arm (ASC therapy). The authors argued that a plausible explanation for this situation is that the research groups lacked experience manipulating cells, and could have deteriorated the cells during the injection. In addition, significant differences were found in the complexity of patients enrolled in different hospitals. After this experience, some long-term results have been published with modest results [42-44], though others in very complex clinical settings have yielded sufficient results with limited and harmless surgery [45-47]. In conclusion, overall healing rates of patients treated with ASCs range from 30% to 60%. This may be considered a poor result, though it would nonetheless be worth the effort if we achieve one healing in a patient with a complex perianal fistula sometimes avoiding a permanent ostomy.

Ongoing clinical trials

There are 16 ongoing trials registered on the clinicaltrials.com database (Table 3 and Table 4). Nine are indicated as "unknown status" and are excluded from the analysis. Seven are in ongoing status and one has been completed and is pending of publication. Since the last review [33], 5 new trials have been registered. Three of these new clinical trials use adipose-derived stromal cells, 2 delivered in situ and one using a cell-coated plug. The other 2cells derived from bone marrow and allogeneic umbilical cord.

Discussion

Wound healing is the basis of surgery, and to orchestrate this process, MSCs could be helpful due to their potent anti-inflammatory and immunomodulatory effects [31]. Their ability to migrate to the site of injury has also been described [16], facilitating these immunomodulatory and regenerative properties in the exact place where they are required.

MSCs were initially described as a bone-marrow-derived mononuclear cell population with a fibroblast-like morphology. Later, MSCs were reported to be found in many different tissues including fat. Although the mechanisms underlying the effects of MSCs have not been clearly defined, MSCs interact with several steps of inflammation during the healing process [15].

Even a simple perianal fistula remain poses a real surgical challenge due to the high rate of recurrence and the possibility of fecal incontinence after surgery [3]. In a more complex setting, multiple operations and incontinence is the rule, and permanent ostomy is eventually needed.

The frustrating outcomes seen in perianal fistula treatment can be explained in part by a deficient and disorganized cell supply during the wound-healing process, and also by the chronic inflammation of the fistula tract. In the case of perianal fistula associated with CD, the problem becomes worse due to the inflammatory basis of the disease, making healing more inefficient [8].

Correct epithelization of the surface of the wound is vital in this case. Suboptimal epithelization may result in the following: i) the inflammation may destroy deeper tissues, thereby expanding the fistula tracks; ii) inflammation may epithelize the fistula track itself leading to perpetuation of the fistula; and iii) fibrosis and scars may deform the shape of the anus, leading to incontinence [14]. In this scenario, cells could be helpful in orchestrating the healing process.

Preclinical studies conducted by different groups in animal disease models have already demonstrated that MSCs are efficient in improving the healing process. All these studies have demonstrated that MSCs area safe *in vivo* therapy [30].

Cells have yielded promising results in preclinical studies directed at treating perianal fistula, some approaches have begun clinical development and, and the number of registered clinical trials has been increasing every year since 2003. In 2015, the most advanced programs, which involve CD and fistulous disease, have reached phase III of development.

The results in phase-III randomized clinical studies have shown, however, that the therapeutic efficacy of MSCs in humans is still modest (40%) [27]; we are currently awaiting the results of ongoing large-scale clinical trials. The safety profile evidenced in these trials and long-term analyses have once again demonstrated the safety of MSCs in clinical settings [31]. Nevertheless, a deeper analysis of this result reveals that no other treatment achieves better results in complex perianal fistula with or without CD, and successful treatment of even one patient merits recognition.

In our experience we prefer adipose-derived MSCs due to their quality and quantity. One liposuction treatment obtains enough cells to make the first injection in raw form on the same day of the extraction, and the second injection may use expanded MSCs if needed; if not, they are cryopreserved.

Combination with any other surgical (limited or more reconstructive) and/or other medical treatments may potentiate both approaches.

Table 2: Published clinical experiences of cell treatments of anal fistula (Part 2). An update of the tables taken from the section "Stem Cell Application in Fistula Disease" [33].

Investigator	Intervention model	Masking	Procedure	Enrolled	Number of treated patients	Healed	Follow up (months)	Recurrence	SAE
García-Olmo *et al.* [34]	Single arm	Open label	Closure of IO. Without fibrin glue. Injection in site	1	1	1	3	0	0
García-Olmo *et al.* [41]	Single arm	Open label	Cells resuspended in fibrin glue. Injection in site	9	9	6 (66%)	12	Not specified	0
García-Olmo *et al.* [25]	Two arms: fibrin glue, fibrin glue + ASCs	Open label	Closure of IO. Injection in site	50 (35 with Crohn's disease)	Fibrin glue: 25 Fibrin glue + ASCs: 24	Fibrin glue: 3 Fibrin glue + ASCs: 17 (70%)	12	Fibrin glue: 0 Fibrin glue + ASCs: 2	4 (only one related to fibrin glue, others not related)
García-Olmo *et al.* [40]	Single arm	Open label	Closure of IO. Without fibrin glue. Injection in site	1	1	1	36	1	0
Ciccocioppo *et al.* [39]	Single arm	Open label	Four injections in site	12	10	7 (70%)	12	0	0
Herreros *et al.* [27]	Three arms: fibrin glue, ASCs, fibrin glue + ASCs	Double blind (subject, outcomes assessor)	Closure of IO. Injection in site	214	ASCs: 64 Fibrin glue + ASCs: 60 Fibrin glue: 59	ASCs: 27 (42%) Fibrin glue + ASCs: 24 (40%) Fibrin glue: 23	6	ASCs: 0 Fibrin glue + ASCs: 4 Fibrin glue: 0	4 unrelated to study treatment
Herreros *et al.* [27]	Three arms: fibrine, ASCs, fibrin glue + ASCs	Double blind (subject, Outcomes Assessor)	Closure of IO. Injection in site	135	Not specified	ASCs: 57% Fibrin glue+ ASCs: 52.4% Fibrin glue: 37.3%	12	Not specified	1 unrelated to study treatment
Guadalajara *et al.* [42]	Two arms: fibrin glue, fibrin glue + ASCs	Open label	Closure of IO. Injection in site	34	Fibrin glue: 13 Fibrin glue + ASCs: 21	Fibrin glue: 3 Fibrin glue + ASCs: 10 (47%)	38	Fibrin glue: 1 Fibrin glue + ASCs: 5	0
Borowski *et al.* [35]	Single arm	Open label	Flap + Injection in site	3	3	3	3	0	0
de la Portilla *et al.* [37]	Single arm	Open label	Closure of IO. Without fibrin glue. Injection in site	34	24	9 (37%)	4	Not specified	2 unrelated to study treatment
Cho *et al.* [38]	Single arm: Dose escalation study	Open label	Closure of IO. Fibrin glue. Injection in site	10	9	3 (33%)	8	0	0
Lee *et al.* [36]	Single arm	Open label	Closure of IO. Fibrin glue. Injection in site	50	43	27 (62%)	12	3	1 unrelated to study treatment
Cho *et al.* [43]	Single arm	Open label	Closure of IO. Fibrin glue. Injection in site	41	41	26 (63%)	24	5	0
Ciccocioppo *et al.* [44]	Single arm	Open label	Four injections in site	8	8	2 (25%)	72	0	0
García-Olmo *et al.* [45]	Single arm	Open label	Flap + Injection in site	10	10	6 (60%)	12	0	0
Molendijk *et al.* [24]	Four arms: control group, ASCs different dose	Double blind	Closure of IO. Injection in site	21	15	7 (46%)	6	Not specified	0
Borowski *et al.* [46]	Single arm	Open label	Closure of IO. Injection in site	7	7	5 (71%)	46	1	1 unrelated to study treatment
Park KJ. [47]	Two arms: ASCs different dose	Open label	Injection in site	6	6	3	8	0	0

Table 3: Ongoing clinical trials using cells for treatment of anal fistula (Part 1). An update of the tables taken from the section "Stem Cell Application in Fistula Disease". [33] Source: Clinicaltrials.gov

Trial code	Condition	Sponsor	Investigator	Study start date	Location
NCT00999115	Recto-vaginal fistula in Crohn's disease	Instituto de Investigación Hospital Universitario la Paz	García-Olmo D	2009	Spain
NCT01541579	Perianal fistula in Crohn's disease	Tigenix	Panes J	2012	Europe, Israel
NCT00482092	Crohn's disease (reduction in number of draining fistulas)	Osiris Therapeutics	Custer L	2007	USA, Australia, Canada, New Zealand
NCT01803347	Complex perianal fistula without Crohn's disease	Instituto de Investigación Sanitaria Fundación Jiménez Díaz	García-Olmo D	2013	Spain
NCT02403232	Perianal fistula in Crohn's disease	Papa Giovanni XXIII Hospital	Bertoli P	2015	Italy
NCT01915927	Perianal fistula in Crohn's disease	Mayo Clinic	Faubion WA	2015	USA
NCT01874015	Perianal fistula in Crohn's disease	Royan Institute	Gourabi H	2013	Iran
NCT02000362	Crohn's disease	Asan Medical Center	Yang SK	2013	Korea
NCT02520843	Perianal fistula in Crohn's disease	Assistance Publique Hopitaux de Marseille	Grimaud JC	2015	France
NCT02677350	Perianal fistula in Crohn's disease	Joshua M Hare, University of Miami	Kerman D	2016	USA

Table 4: Ongoing clinical trials using cells for treatment of anal fistula (Part 2). An update of the tables taken from the section "Stem Cell Application in Fistula Disease". [33] ASCs: adult stem cells. Source: Clinicaltrials.gov

Trial code	Cell Source	Expanded	Cells number	Status	Phase	Intervention model	Masking	Estimated enrollment
NCT00999115	Allogeneic fat	Yes	2 ×10e7, when no effect 4 ×10e7	Completed. Publications not provided	1.2	Single arm	Open label	10
NCT01541579	Allogeneic fat	Yes	12 × 10e7	Ongoing, not recruiting	3	Two arms: (ASCs, placebo)	Double blind	208
NCT00482092	Allogeneic bone marrow	Yes	120 × 10e7 or 60 × 10e7 cells infused in four visits	Ongoing, not recruiting	3	Three arms: Placebo, high dose and low dose	Double blind	270
NCT01803347	Autologous fat	Yes	10 × 10e7, additional dose when no effect	Recruiting	3	Two arms: ASCs + fibrin glue, fibrin glue	Double blind	80
NCT02403232	Autologous fat	No	Not specified	Recruiting	2	Single arm ASCs + infliximab	Open label	10
NCT01915927	Autologous fat	Yes	2 × 10e7	Recruiting	1	Stem-cell-coated plug	Open label	20
NCT01874015	Autologous bone marrow	Yes	Dose not specified. 4 injections	Recruiting	1	Two arms: ASCs + fibrin, ASCs + fibroblast + fibrin	Single blind	10
NCT02000362	Allogeneic umbilical cord	Yes	5 × 10e7 or 1 × 10e8	Recruiting	1.2	Two arms: low dose, high dose	Open label	24
NCT02520843	Autologous fat	No	Not specified	Recruiting	1.2	Single arm	Open label	10
NCT02677350	Allogeneic bone marrow	Yes	2 × 10e7 divided in 10 inyections of 2 millon	Recruiting	1	Single arm	Open label	20

According to current data we can say that MSC therapy may become a clinical reality for the treatment of complex perianal fistula in the near future.

Moreover, in the field of cell therapy a new generation of cell-based drugs is being developed to improve the therapeutic efficacy of conventional cell-based treatments such as the engineered cells or cells mixed with scaffolds [48].

Conclusions

Surgical wound healing is the most fundamental aspect of surgery. MSCs could be helpful in orchestrating this process due to their potent anti-inflammatory and immunomodulatory effects. Successful treatment of perianal fistula is exceedingly difficult, and even small tract fistulas may have difficulty healing. This difficulty can be explained due to the impairment of the healing process provoked by this condition. This situation is exacerbated in CD. Multiple failed operations are common. Although published results may seem discrete, no other techniques have shown better results nor have the same positive safety profile. Combination with any other surgical (limited or more reconstructive) or medical treatments may potentiate both types. According to current data, we can say that MSC therapy may become a clinical reality for the treatment of complex perianal fistula in the near future.

Financial and Competing Interests Disclosure

UAM and Cellerix SL/Tygenix TAU, share patents rights in cell products. García-Olmo is a member of the scientific advisory board of Tygenix. Damian García-Olmo and Mariano García-Arranz are inventors in two patents related to cell products entitled "Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue" (10157355957US) and "Use of adipose tissue-derived stem cells in treating fistula" (US11/167061).

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