Adipose-Derived Stem Cells - A Promising Method of Therapy for Perianal Fistula: A Traditional Review

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ABSTRACT

Background: Perianal fistula is a highly exhausting and difficult - to - treat condition and might negatively impact patients' quality of life. Recently, the application of mesenchymal stem cells has shown notable encouraging results in treating perianal fistula. Previous studies have shown that adult stem cells isolated from adipose tissue imply a subset of pluripotent mesodermal stem cells with a potential differentiation in myogenic, audiogenic, and chondrogenic types of cells. Additionally, human adipose-derived stem cells being multipotent cells, are capable of self-renewal and differentiation and have become apparent as crucial regulators of an immune response.
It is well known that mesenchymal stem cells exist in almost all tissues and probably reduce inflammation through their intrinsic immunomodulatory properties; however, the exact mechanism of action in treating perianal fistula still under development.

Aim: The present review aims to analyze the published scientific literature on adipose-derived MSCs in treating perianal fistula and clinical outcomes.

Method: A literature search in PMC, PubMed, Google, and Google Scholar was carried out using the following keywords: "perianal fistula," "stem cells and perianal fistula" "adipose derived stem cells in perianal fistula," "mechanism of action of adipose derived stem cells" Study selection was in the language (English only), model (humans only), open accesses, and all types of studies were included as long as they were relevant to our study.

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Keywords: Anal Fistula, Adipose Derived Stem Cells, Perianal Fistula, Stem Cells, Mesenchymal Stem Cells.

1. Introduction:

The prevalence of fistula-in-ano is unknown; however, the incidence ranges from 26% to 38% of a fistula-in-ano developing from an anal abscess (Poggio JL, 2020). And about 35-50% of adults with Crohn's disease will develop a perianal fistula at some point in their disease journey (IBDVisible Blog, 2020). Additionally, studies showed that the prevalence of fistula-in-ano is 8.6 cases per 100,000 population (Poggio JL, 2020). In men, the prevalence is 12.3 cases per 100,000 population and 5.6 cases per 100,000 population in women (Poggio JL, 2020). The male-to-female ratio is around 1.8:1, while the mean patient age is 38.3 years (Poggio JL, 2020).

1.1 What is an anal fistula?

An anal fistula (fistula-in-ano) is frequently the result of a previous or current anal abscess which occurs in up to 40% of patients with abscesses. An anal fistula is an epithelialized tunnel that connects a clogged gland inside the anal canal to the skin from the outside (American Society of Colon and Rectal Surgeons, 2020).

1.2 What causes an anal fistula?

Clogged anal glands and anal abscesses are the dominant causes of an anal fistula (IBDVisible Blog, 2020; American Society of Colon and Rectal Surgeons, 2020). Additionally, much less common conditions that can cause an anal fistula include: Crohn's disease, trauma, radiation (treatment for cancer), sexually transmitted diseases,

diverticulitis, cancer, chronic diarrhea, colitis, tuberculosis, inflammatory bowel disease (IBD), steroid therapy, HIV infection (Poggio JL, 2020; American Society of Colon and Rectal Surgeons, 2020).

1.3 The symptoms of an anal fistula:

A review of symptoms may reveal abdominal pain, weight loss, change in bowel habits in patients with a fistula-in-ano (Poggio JL, 2020). Additionally, patients may complain of pain, swelling around the anus, frequent anal abscesses, and foul-smelling drainage (pus)or bloody discharge from an opening around the anus (Poggio JL, 2020, American Society of Colon and Rectal Surgeons, 2020). The pain may diminish after the fistula drains (Poggio JL, 2020). The drainage can cause irritation and itching of the skin around the anus, along with painful bowel movements (Poggio JL, 2020, American Society of Colon and Rectal Surgeons, 2020). Some patients may complain of fever, chills, and a general feeling of fatigue (Poggio JL, 2020, American Society of Colon and Rectal Surgeons, 2020). The symptoms showed in patients with perianal fistula are demonstrated below in Figure 1.

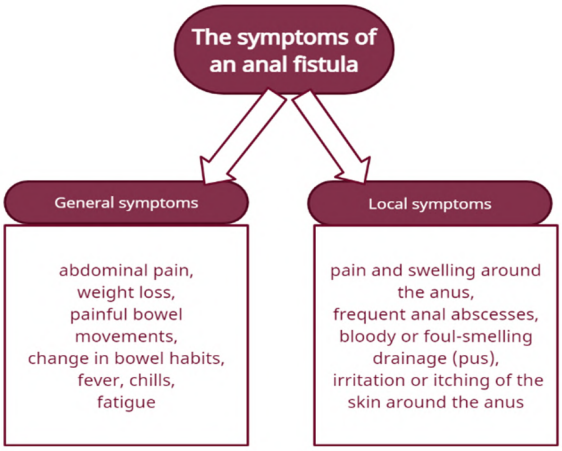

Figure 1. The symptoms showed in patients with perianal fistula.

Methods of Diagnosis of Anal Fistula:

Physical findings are crucial for diagnosis (Poggio JL, 2020). The examiner should observe the entire perineum as a spontaneous discharge of blood or pus via the external opening may be visible on digital rectal examination (Poggio JL, 2020).

Digital rectal examination may detect a fibrous tract or cord under the skin or an acute inflammation that is not drained (Poggio JL, 2020). The sphincter tone and voluntary squeeze pressure should be evaluated to determine whether preoperative manometry is needed (Poggio JL, 2020). Anoscopy is demanded to identify the internal opening, whilst proctoscopy is indicated in patients with Crohn's disease (Poggio JL, 2020).

Besides, other methods may be used to help with the diagnosis. These include fistulography- a technique that involves an injection of contrast via the internal opening, followed by radiographic images to outline the course of the fistula tract (Poggio JL, 2020). However, the accuracy is low, which has been reported to range from 16% to 48% (Poggio JL, 2020).

Endoanal or endorectal ultrasonography involves the passage of an ultrasound transducer into the anal canal, which is 50% better than physical examination alone in detecting an internal opening that is difficult to localize (Poggio JL, 2020).

Magnetic resonance imaging (MRI) is becoming the study of choice, as MRI shows an 80-90% similarity with operative findings (Poggio JL, 2020). MRI also outlines the extent and secondary ramifications of the fistula tract as well as detecting the anal and cutaneous openings. Additionally, MRI can differentiate isolated active inflammation from associated fistulous tracts, which can be confusing on palpation (Balcı et al., 2019).

1.4 How is an Anal Fistula Treated?

Treatment of anal fistula depends on the complexity and location of the anal fistula. The main goal while repairing a fistula is a complete treatment to prevent recurrence and to protect the sphincter muscles, as damage of them can lead to fecal incontinence (Mayo Clinic ,2020; NHS, 2019). Surgery is usually required to treat an anal fistula as they usually do not heal by themselves (NHS, 2019).

The main options are outlined here:

Fistulotomy - The most frequent type of surgery applied for the treatment of anal fistulas [(Poggio JL, 2020; Mayo Clinic, 2020; NHS, 2019). This includes cutting with a knife or electrocautery the whole length of the fistula tract, along with curettage to remove granulation tissue in the tract base, which helps it to heal as a flat scar [(Poggio JL, 2020; Mayo Clinic, 2020; NHS, 2019). This technique is useful for 85-95% of primary fistulas (Poggio JL, 2020).

Seton Placement - If the fistula involves a significant portion of the anal sphincter muscle, the surgeon may insert a seton [(Poggio JL, 2020; Mayo Clinic,2020; NHS, 2019). A silk or latex string (seton) is placed into the fistula for a few weeks to help drain the infection and heal, avoiding the need to cut the sphincter muscles (Poggio JL, 2020; Mayo Clinic, 2020; NHS, 2019). Loose setons drain but do not cure them, while tighter setons cut through the fistula slowly and promote fibrosis. The success rates for cutting setons are around 82-100%; though, long-term fecal incontinence rates can exceed 30% (Poggio JL, 2020).

Advancement Mucosal Flap - This procedure is used if the fistula passes through the anal sphincter muscles, and the fistulotomy carries a high risk of causing incontinence (NHS, 2019). A muco-muscular flap is used to cover the repair of the internal opening of the perianal fistula

[(Poggio JL, 2020; NHS, 2019). This is done in a one-stage procedure with minimal sphincter damage; however, a poor success rate is noted in patients with Crohn's disease and acute infection (Poggio JL, 2020).

Plugs and Adhesives - After cleaning the channel of the fistula and stitching the internal opening, a special glue made from a fibrous protein (fibrin)or a plug of collagen protein is then injected through the fistula's external opening (Mayo Clinic, 2020). Another option is the insertion of a cone-shaped plug of a bioprosthetic plug made from animal tissue (NHS, 2019) This choice is less effective than fistulotomy, and the results may not be long-lasting (NHS, 2019). Other relatively new options are ligation of the inter-sphincteric fistula tract (LIFT) and Video-Assisted Anal Fistula Treatment (VAAFT).

Ligation of the inter-sphincteric fistula tract (LIFT) - This procedure is a treatment option for complex trans-sphincteric fistulas, where a fistulotomy can be followed by fecal incontinence (Poggio JL, 2020; (Mayo Clinic, 2020; NHS, 2019). A cut must be done in the skin above the fistula, and moving the sphincter muscles apart, avoiding cutting them (NHS, 2019). Then fistula is sutured at both ends and cut open, so it lies flat (NHS, 2019). Because the LIFT procedure is relatively new, it has not been extensively researched (Poggio JL, 2020).

Video-Assisted Anal Fistula Treatment (VAAFT) - The essential characteristic of this technique includes the ability to view the fistula from the inside so that it can be eradicated under direct vision using a fistuloscope (Meinero & Mori, 2011). Firstly, destruction of the fistula from the inside is done, along with cleansing of the fistula tract, then closure of the internal opening is done (Meinero & Mori, 2011). A novel treatment option for improving regeneration and/or repair of damaged tissues in a particularly unfavorable environment for wound healing is the application of stem cell therapy (Panes, 2016).

Stem Cell Therapy - The stem cells used for the treatment of perianal fistulas are mesenchymal stem cells (Panes, 2016). For the treatment of perianal fistulas, two sources of mesenchymal stem cells are used: bone marrow and adipose tissue (Panes, 2016). Up to several local injections of mesenchymal stem cells may be used until fistulas close (Panes, 2016). The present study aims to analyze the current studies done on the efficacy of the stem cells therapy, in particular adipose derived mesenchymal stem cells, in the treatment of perianal fistulas.

2. Discution and Review

Stem cells are the body's matrix materials unspecialized cells of the human body able to differentiate into any cell of an organism and have the ability of self-renewal (Zakrzewski et al., 2019)

Mesenchymal stem cells (MSCs) are an example of 'adult' stem cells, which are 'multipotent,' meaning they can produce several types of the specialized cell of the body, but not all types (EuroStemCell, 2020). For example, they can differentiate into fat cells (adipocytes), cartilage cells (chondrocytes), and bone cells (osteoblasts) (EuroStemCell, 2020).

Adipose tissue and bone marrow are derived from the embryonic mesenchyme and contain a stroma. However, adipose tissue can be more easily isolated compared to bone marrow samples (Zuk et al., 2002). Using a lipo-aspiration method, the cell population, called processed lipoaspirate (PLA) cells, can be isolated from adipose tissue (Zuk et al., 2002).

To certify whether PLA cells represent a stem cell population, Zuk and colleagues (2002) conducted a comprehensive analysis of the PLA cells population and adipose-derived stem cells (ADSCs). They noted that PLA cells express several CD marker antigens like those observed on MSCs controls. Additionally, PLA cells showed unique characteristics distinct from that seen in MSCs, like gene expression profiles and differences in the cluster of differentiation (CD) marker (Zuk et al., 2002) The results presented in this study implied that adipose tissue might be an additional source of unique, pluripotent stem cells with multi-germline potential (Zuk et al., 2002). Based on Rehman and colleagues (2004), ADSCs secrete bioactive factors through the paracrine mechanism to promote endogenous cells' proliferation and migration, thereby stimulating angiogenesis, epithelial regeneration, and wound remodeling (Rehman et al., 2004).

ASCs to promote the recovery of wound blood secrete various angiogenesis-related cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factors (PDGF), stromal cell-derived factor 1 (SDF-1), and vascular endothelial growth factor (VEGF) (Rehman et al., 2004). Additionally, ASCs secrete basic fibroblast growth factor (b-FGF), hepatocyte growth factor (HGF), transforming growth factor Alpha (TGF-α), matrix metalloproteinases (MMP), and interleukins (IL)such as IL-6 and IL-8 (Rehman et al., 2004).

Many studies have demonstrated that increases in the proliferation and survival of human adipose stem cells were observed in a hypoxic environment (2%) compared to normoxia (Valorani et al., 2012; Choi et al., 2015; Dai et al., 2005). Rehman and colleagues (2004) also developed that ASCs secretes five times more VEGF under hypoxic conditions than in normoxic conditions, which can significantly increase the proliferation of endothelial cells and reduce apoptosis (Rehman et al., 2004).

Mesenchymal stem cells carry immunomodulatory properties involving three essential steps: 1) migration to places of active inflammation or tissue injury, 2) secreting of anti-inflammatory molecules like TGFβ1, IL-10, HGF, and 3) using paracrine signaling to nearby cells they maintain an anti-inflammatory environment (Dai et al., 2005; Ryan et al., 2007; Horton et al., 2013). MSCs regulate cytokine secretion and manage the function of various immune cell types, such as TGFβ1, lymphocytes,

dendritic cells, and macrophages (Carvello et al., 2019). In Figure 2, are demonstrated presumptive mechanisms of action of stem cells involved in tissue repair and regeneration of perianal fistula.

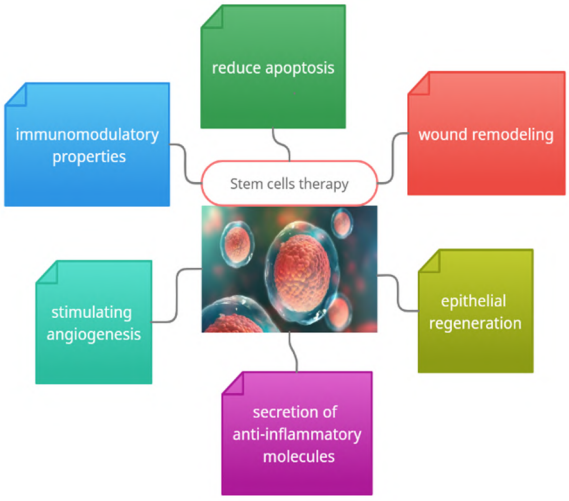

Figure 2. Presumptive mechanisms of action of stem cells involved in tissue repair and regeneration of perianal fistula.

The first report of a clinical trial of stem cell therapy using autologous stem cells obtained from a lipoaspirate was published by Garcia-Olmo and colleagues (2005). They conducted a prospective Phase I clinical trial, testing the suitability and safety of autologous stem cells transplantation in the treatment of fistulas of Crohn's disease (Garcia-Olmo et al., 2005). They inoculated nine fistulas in four patients with autologous adipose tissue-derived stem cells]. One patient was eliminated from the study because of bacterial contamination of cultured cells (Garcia-Olmo et al., 2005). In six fistulas (75%), the external opening was covered with epithelium after eight weeks and was considered healed. In the other two fistulas (25%), there was only incomplete closure of the external opening, with a decrease in output flow. The follow-up period was a minimum of 12 months; the maximum, 30 months (average, 22 months) (Garcia-Olmo et al., 2005)

In 2009 was published the result of phase II of the randomized controlled trial. The trial investigated the effectiveness and safety of ADSCs in the treatment of complex perianal fistulas (Garcia-Olmo et al., 2009). Patients with complex perianal fistulas (Crohn's disease, n = 14, and cryptoglandular origin, n = 35) were treated with intralesional fibrin glue or fibrin glue plus 20 million ASCs. If healing was not achieved at eight weeks, the patients received the second dose of fibrin glue or fibrin glue plus 40 million ADSCs (Garcia-Olmo et al., 2009).

Fistula healing was noted in 17 of 24 patients (71 %) who received ASCs with fibrin glue compared with four of 25 patients (16%) who received fibrin glue alone Garcia-Olmo et al., 2009). The rate of healing was similar in Crohn's and non-Crohn's subgroups. The quality of life was higher in patients who received ASCs compared to those who received fibrin glue alone. The recurrence rate was 17.6% at one-year follow-up (Garcia-Olmo et al., 2009).

After the successful phase I and II of clinical trials, by Garcia-Olmo and colleagues, in 2012 was published the results of phase III by Herreros and colleagues (2012). In a randomized, single-blinded clinical trial, 200 adult patients with complex cryptoglandular perianal fistulas from 19 centers were randomly assigned into three groups (Herreros et al., 2012). Group A (64 patients) received 20 million autologous expanded adipose-derived stem cells, group B (60 patients) 20 million adipose-derived stem cells plus fibrin glue, and group C (59 patients) fibrin glue alone. If the fistula had not healed completely at 12 weeks, the second dose of 40 million stem cells (groups A and B) was administered. Patients were checked at 24 to 26 weeks and one year (long-term follow-up) (Herreros et al., 2012).

The results achieved after 24 to 26 weeks were 39.1%, 43.3%, and 37.3% healing rate in groups A, B, and C, respectively (Herreros et al., 2012). At one year, the healing rates were 57.1%, 52.4%, and 37.3 %, respectively (Herreros et al., 2012).

Additionally, Cho and colleagues (2013) evaluated the safety and suitability of adipose tissue-derived stem cells (ASCs) for the treatment of Crohn's fistula. In this study phase I clinical trial, the patients were enrolled into three dosing groups (Cho et al., 2013). The first three patients in the first group were given 1×10 (7) cells/ml., in the second group were given 2×10 (7) cells/ml., and the third group of three patients was given 4×10 (7) cells/ml (Cho et al., 2013). Two patients in the second group showed complete healing at week eight after injection (Cho et al., 2013). One of three patients enrolled in the third group showed complete healing, and another patient was assessed as partial healing due to incomplete closure of the external opening, with no drainage. All three patients with complete healing at eight weeks showed no recurrence eight months after injections (Cho et al., 2013).

The results of phase II of the clinical trial were published by Lee and colleagues (2013). Forty-three patients with Crohn's fistula were treated with ASCs; 70% (n = 30) were male, and 30% (n = 13) were female (Lee et al., 2013). The quantity of ASCs injected into the fistula depended on the fistula's size. The fistula tract was filled with autologous ASCs in combination with fibrin glue (Lee et al., 2013). Approximately 3×10(7) cells per centimeter length when the fistula's diameter was less than 1 cm were injected, and a double dose of cells was administered when the fistula's diameter was 1 cm< d ≤2 cm (Lee et al., 2013). The second injection of ASCs containing 1.5 times more cells was administered at eight weeks if the healing was not achieved. The analysis showed that complete fistula healing was observed in 27/33 (seven patients – withdrawal) (82%) patients by eight weeks after ASCs

injection (Lee et al., 2013). Of 27 patients, 26 with fistula healing completed an additional observation study for one year, and 23 patients (88%) sustained complete closure (Lee et al., 2013).

At 24 months observational study, 20 of 24 (83%) showed complete closure of the fistula (one patient withdrawal and the data of another two missed) (Cho et al., 2015). Three patients of 26 (11,5%) showed recurrence at 12 months post-treatment, and four of 24 (16,7%) showed recurrence at 24 months post-treatment (Cho et al., 2015).

De la Portilla and colleagues assessed the safety and efficacy of a suspension of expanded adipose-derived allogeneic mesenchymal stem cells (eASCs) in 24 patients with complex perianal fistula of Crohn's disease. The patients were administered intra-lesionally with 20 million eASCs in the fistula tract (De l a Portilla et al., 2013). An additional dose of 40 million eASCs was performed if incomplete closure was observed at 12 weeks and 24 weeks (De la Portilla et al., 2013). At week 24, 69.2 % of the patients had a reduction in the number of draining fistulas, 56.3 % of the patients achieved complete closure of the fistula, and 30 % of the cases presenting full closure of all existing fistula tracts (De la Portilla et al., 2013).

A multicenter, open-label, dose-escalation pilot study had been performed by Park and colleagues. The study evaluated the safety and feasibility of allogeneic ASCs to treat perianal fistula in Crohn's disease (Park et al., 2016). In the group-1, three patients received 1×10(7) cells/ml based on the fistula tract's size [27]. Four weeks later, the next three patients from group-2 were administered 3×10(7) cells/ml. Patients who attended the eight-week assessment were followed for an additional six months (Park et al., 2016)].

Two patients in group-1 achieved complete closure of the fistula at four and six months, and one patient in group-2 achieved complete closure at eight weeks. The closure was sustained up to eight months posttreatment in all three of those patients (Park et al., 2016).

Panés and colleagues. performed a randomized placebo-controlled trial, a double-blind study at 49 hospitals in Europe and Israel. The study comprising 212 patients with Crohn's disease and treatment-refractory, draining, complex perianal fistulas (Panés et al., 2017).

Patients were randomly assigned (1:1) into two groups, receiving a single local injection of 120 million allogeneic expanded adipose-derived stem cells (Cx601) cells or placebo (Panés et al., 2017). Combined remission in 51.5% of patients given Cx601 vs. 35.6% of controls was achieved at 24 weeks follow-up, while at week 52, combined remission achieved (56.3%) vs. controls (38.6%), and clinical remission 59.2% vs. 41.6% of controls (Panés et al., 2017).

Herreros and colleagues (2019) evaluated the efficacy of compassionate use of ASCs in the treatment of patients who had previously multiple surgical interventions that had failed. The intervention consisted of surgery (with the closure of the internal opening with a surgical flap), followed by stem cell injection (Herreros et al., 2019). Three types of cells were used during the treatment: stromal vascular fraction (SVF), autologous expanded adipose-derived, or allogenic adipose-derived stem cells (Herreros et al., 2019). SVF was administered in 31/52 (60%) cases, allogenic expanded adipose-derived stem cells (Allo-eASC) were used in 12/52 (23%), and autologous expanded adipose-derived stem cells (Au-eASC) were used in 9/52 (17%) [29]. Doses of ASCs administered ranging from one to 210 million with a mean of 48 million (Herreros et al., 2019).

Forty-five patients received the treatment; since some of them received additional doses, 52 cases were considered (42 fistula-in-ano, seven rectovaginal fistulas, one urethrorectal fistula, one sacral fistula, and one hidradenitis suppurativa (Herreros et al., 2019). Eighteen patients with fistula-in-ano, were Crohn's-associated and 24 cryptoglandular origins (Herreros et al., 2019). Forty-nine cases (94.2%) showed a partial response at 6.5 weeks of follow-up, while 24 subjects (46.2%) healed in a mean time of 5.5 months. At one-year follow-up, all patients cured remained healed (Herreros et al., 2019).

Additionally, Garcia-Arranz and colleagues conducted a clinical trial to determine the safety and efficacy of autologous ASCs for the treatment of cryptoglandular fistula (Garcia-Arranz et al., 2020). They conducted a multicenter, randomized, single-blind clinical trial in which 57 patients participated. Forty-four patients were categorized as the intent-to-treat group [30]. Following a deep curettage of tracks and closure of internal openings, 23 patients (group A) received 100 million ASCs plus intralesional fibrin glue, and 21 (group B) received intralesional fibrin glue (Garcia-Arranz et al., 2020).

The patients in whom the fistula had not healed at 16 weeks posttreatment received additional treatment (Garcia-Arranz et al., 2020). Patients were evaluated at one, four, 16, 36, and 52 weeks and two years after treatment [30]. After 16 weeks, the healing rate was 30.4% (group A) vs. 42.8% (group B), rising to 55.0% and 63.1%, respectively, at 52 weeks (Garcia-Arranz et al., 2020). At two years after treatment, the healing rate remained at 50.0% (group A) and 26.3% (group B (Garcia-Arranz et al., 2020).

3. Limitations

While we were searching the information for this review, there were some limitations. Our data was primarily obtained from articles with free full access and written in English language only; thus, relevant articles of closed access and written in other languages may have been skipped. This review article is a traditional review and, therefore, does not follow the standard Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews.

In Table 1 is shown the summary of results of previous clinical studies using ADSCs.

Authors	Year	Type of the study	Number of patients	Treatment used, dose	Results achieved
Garcia-Olmo et al.	2005	A prospective Phase I clinical trial	5 Pt with Crohn's fistulas, 1- excluded due to bacterial contamination of cultured cells, 9 fistulas in total	Autologous ADSCs- Local injection	6-fistulas (75%), at 8 weeks were considered healed. 2-fistulas (25%), incomplete closure of the external opening, with a decrease in output flow
Garcia-Olmo et al.	2009	Phase II of the randomized controlled trial	Complex perianal fistulas (Crohn's disease, n = 14, Cryptoglandular origin, n = 35) 24 Pt received ASCs+ fibrin glue 25 Pt received fibrin glue alone	Autologous-ADSCs. Intralesional fibrin glue or fibrin glue plus 20 million ASCs If healing was unsuccessful at 8 weeks, the second dose of fibrin glue or fibrin glue plus 40 million ASCs	17 Pt (71 %) of 24 patients who received ASCs in addition to fibrin glue-healed 4 Pt (16 %) of 25 Pt who received fibrin glue alone The recurrence rate in Pt treated with ASCs was 17.6% at 1-year follow-up
Herreros et al.et al.	2012	Phase III, randomized, single-blinded clinical trial	200 Pt with complex cryptoglandular perianal fistulas Divided into 3 groups A- Autologous expanded ADSCs B- ADSCs + fibrin glue C-fibrin glue	Group A (64 Pt) received 20 million Autologous expanded ADSCs, group B (60 Pt) 20 million ADSCs + fibrin glue, group C (59 Pt) fibrin glue alone. If not healed at 12 weeks- second doses of 40 million stem cells (groups A and B)	After 24 to 26 weeks healing rate was: Group A- 39.1%, Group B- 43.3% Group C- 37.3% At one year, the healing rate was Group A- 57.1%, Group B- 52.4% Group C- 37.3 %
Cho et al.	2013	Phase I clinical trial	3- dosing groups 9 Pt with Crohn's fistula	Autologous ASCs 1st group, 3 Pt 1×10 (7) cells/ml, 2nd group,3 Pt 2×10 (7) cells/ml 3rd, 3 Pt, 4×10 (7) cells/ml	2 Pt in the second group, showed complete healing 1 Pt of the third group, showed complete healing 1 Pt (3rd) partial healing due to incomplete closure of the external opening, with no drainage

In Table 1 is shown the summary of results of previous clinical studies using ADSCs.

Authors	Year	Type of the study	Number of patients	Treatment used, dose	Results achieved
Lee et al. **Follow up at 2 years: Cho et al.**	2013 2015	Phase II of the clinical trial	43 Pt with Crohn's fistula	Autologous-ADSCs Intralesional injection of ASCs+ fibrin glue 3 × 10(7) cells per cm length when the fistula's diameter was <1 cm 6 × 10(7) cells per cm length diameter when was 1 cm< d ≤2 cm A second injection containing 1.5 times more cells, at 8 weeks if incomplete healing	27/33 (7 Pt – withdrawal) (82%) Pt by 8 weeks- complete healed 23 of 26 Pt (88%)- complete closure at 1-year follow-up At 2 years follow-up 20 of 24 (83%)- complete closure of the fistula (1 Pt withdrawal, the data of another 2 missed) 3 Pt of 26 (11,5%)- recurrence at 12 months post-Tx, 4 of 24 (16,7%) - recurrence at 24 months post-Tx
De la Portilla et al.	2013	A multicenter phase I/II (a) clinical trial	24 Pt with complex perianal fistula of Crohn's disease	Expanded eASCs Allogeneic-ADSCs, Intralesional with 20 million eASCs 40 million eASCs if fistula closure was incomplete at 12 weeks	At week 24- 69.2 % Pt had a reduction in the number of draining fistulas, 56.3 % Pt achieved complete closure of the treated fistula, 30 % Pt complete closure of all existing fistula tracts
Park et al.	2016	A multicenter, open-label, dose-escalation pilot study	6 Pt with perianal fistula in Crohn's disease 2- study groups	Allogeneic ASCs group-1, 3 Pt received 1×10(7) cells/ml based on the fistula tract's size group-2, 3 Pt received 3×10(7) cells/ml, after 4 weeks	group-1 achieved complete closure of the fistula at 4 and 6 months group-2, 1 Pt achieved complete closure at eight weeks
Panés et al.	2017	A randomized placebo-controlled trial, a double-blind study	212 Pt with Crohn's, draining, complex perianal fistulas Pt dived into 2 groups: allogeneic expanded adipose-derived stem cells (Cx601) cells or placebo (control)	A single local injection of 120 million allogeneic expanded ADSCs (Cx601) cells or placebo (control)	At week 24, was reported (combined remission in 51.5% of patients given Cx601 vs. 35.6% of controls, while at week 52, combined remission achieved (56.3%) vs. controls (38.6%), and clinical remission (59.2% vs. 41.6% of controls

In Table 1 is shown the summary of results of previous clinical studies using ADSCs.

Authors	Year	Type of the study	Number of patients	Treatment used, dose	Results achieved
Herreros et al.	2019		45 Pt-52 cases 42 fistula-in-ano, 7-rectovaginal fistulas, 1-urethrorectal fistula, 1- sacral fistula, 1- hidradenitis suppurativa Fistula –in-ano: 18 Crohn's-associated and 24 cryptoglandular	SVF was administered in 31/52 (60%) cases, Allo-eASC were used in 12/52 (23%), Au-eASC were used in 9/52 (17%) Doses from 1 to 210 million with a mean of 48 million, some had repeated doses	49 cases (94.2%) showed a partial response at 6.5 weeks 24 cases (46.2%) healed in a mean time of 5.5 months. At 1-year follow-up, all Pt cured remained healed
Garcia-Arranz et al.	2020	A randomized single-blind clinical trial	57 Pt with cryptoglandular fistula 23 Pt (group A): ASCs + intralesional fibrin glue 21 Pt (group B): intralesional fibrin glue	Autologous ASCs (group A) received 100 million ASCs plus intralesional fibrin glue; not healed at 16 weeks-repeated treatment (group B): intralesional fibrin glue alone	At- 16 weeks, the healing rate was 30.4% (group A) vs. 42.8% (group B) At 52 weeks- 55.0% and 63.1% At 2 years the healing rate remained at 50.0% (group A) and 26.3% (group B)

Summary of results of previous clinical studies using ADSCs in patients with perianal fistula.
Pt- patients, ADSCs- Adipose derived stem cells ASCs- adipose tissue-derived stem cells, Allo-eASC- Allogenic expanded adipose derived stem cells, Au-eASC- autologous expanded adipose derived stem cells, eASCs-, SVF-stromal vascular fraction

3. Conclusion

The management of perianal fistula is controversial, and available treatment options have a relatively limited success rate. Mesenchymal stem cell treatment is an attractive therapeutic strategy for patients with perianal fistulas in CD and fistula of cryptoglandular origin. The application of adipose-deriver mesenchymal stem cells (ADSCs) is greater than that of bone marrow-derived mesenchymal stem cells (BM-MSCs) in regenerative medicine. ADSCs require an effortless technique for isolation when compared to BM-MSCs.

The use of adipose-derived MSCs is promising; however, the transplant in the luminal region should be more investigated. The exclusive injection of MSCs in the perianal fistula is better investigated than treatment together with other products, which should be better investigated and used with caution following present standard techniques in clinical studies.

No absolute cell dosage and administration procedure have been consistently identified in the trials to date. The cell dosage has ranged from one to 210 million cells delivered, with inconsistent protocols regarding repeated injection and various delivery methods, including direct injection, injection with fibrin glue, and delivery on a fistula plug. Further clinical trials are demanded to study the required dose and the route of delivery of the MCSs, which will help to answer these questions. The mechanism which is involved in fistula healing should also be better explored. The mechanism of action of MSCs is still not fully understand. More detailed research is required, which will help doctors and scientists treat patients with perianal fistula more effectively.

Further studies are mandatory to determine the impact of MSCs administration in complex fistulas with multiple fistula tracts and patients with active inflammation.

Conflict of Interest

All authors declare no competing interests.

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