Review article

Stem cell therapy in anal fistula: a mini review

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Abstract: When an abscess is drained, either spontaneously or surgically, the pathway to the infection remains and is lined with epithelial tissue and leads to the formation of anal fistulas. The treatment of fistulas remains a serious challenge for colorectal surgeons with varying degrees of success because extensive surgery to complete control complex fistulas results in fecal incontinence. One of the most recent non-surgical methods is cell therapy, clinical trials for which are ongoing in the third phase. In this method, stem cells with different sources are used to control inflammation and replace the lost tissue in the fistula tract. Therefore, the purpose of this narrative review is to review cell therapy in treatment of anal fistulas along with its weaknesses and strengths. Finally this review of existing studies for anal fistulas suggests that until now, only two sources of stem cells, in both allogeneic and autologous forms, have been used in clinical trials for treatment of anal fistulas: adipose tissue and bone marrow (mainly adipose tissue) and except for the two Phase III clinical trials in 2012 and 2016, all clinical trials in this field were conducted in phase one and two with the aim of determining the safety and efficacy of these cells.

Keyword: Stem Cells; Cell Therapy; Anal Fistula

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1. Introduction

Most abscesses in the perineum originate from one of the anal glands. Obstruction of these glands causes excessive bacterial growth and ultimately creates an abscess located in the space between the sphincter (external and internal sphincter) (1). These abscesses have several paths to go out. Most of them go down to the anoderm (perineal abscesses) or through the thickness of the external anal sphincter (EAS) and pass into the ischiorectal space. Few will open up to the supralvator space. When an abscess is drained, either spontaneously or surgically, the pathway to the infection remains and is lined with epithelial tissue and leads to the formation of anal fistulas. Approximately 60% of abscesses will have such a fate (2). Out of every 10,000 people, 1.2 to 2.8 people are diagnosed with anal fistula (3). A typical fistula consists of a primary orifice (internal) and a secondary orifice (external), but in some cases the tract may become obstructed along its path and remain as a sinus so the perineal sinuses should be considered as a type of perineal fistula (4). The most common classification of fistulas can be attributed to the suggestion of Parks that the fistula is classified to: Intersphincteric fistula (fistula tract in the space between the external and internal anal sphincters toward the perineum), transsphincteric fistula (fistula tract passes through the thickness of the EAS and opens into the ischiorectal space), suprasphincteric fistula (fistula tract passes through the top of the EAS and opens in the perineum) and extrasphincteric fistula (fistula tract passes above the external sphincter and opens in the perineum without the involvement of the sphincter) (5). The aim of the surgery of fistula is to remove the fistula, maintain the integrity of the sphincter and prevent the recurrence of the infection as much as possible (6). Most cases of fistulas are very clear and simple and can be easily treated with surgery, for example, when the fistula tract passes through the lower third of the sphincter, it can be treated with a simple lay-open. But more than 30 percent of the fistulas are complex, and they are very difficult to treat via common treatments (5). Limited surgical procedures (in order to maintain the integrity of the sphincter) are associated with a high rate of recurrence, and extensive surgery to complete

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control complex fistulas results in fecal incontinence (due to damage to the EAS) (7). The treatment of complex fistulas remains a serious challenge for colorectal surgeons with varying degrees of success (8). The treatment of perinatal fistulas without controlling inflammation around the fistula tract and restoring lost tissue is almost impossible. Today, the use of stem cells alone or in combination with other strategies has been highly considered by researchers as an effective strategy for reconstruction and replacement of damaged tissues in various diseases (9-17), including fistulas. Therefore, the purpose of this narrative review is to review cell therapy in treatment of anal fistulas along with its weaknesses and strengths.

2. Epidemiology

The actual incidence of fistulae in the general population is uncertain. In a study aiming to determine the actual prevalence of anorectal fistula in Europe with data from four European countries (Germany, Spain, Italy and the United Kingdom), this rate was reported to be between 12 and 28 per 100,000 people (18). Its prevalence is higher in men than in women. In the Sainio study, the ratio of men with fistula to women was reported as 1: 1.8 (18). According to the results of existing studies, its incidence is higher in the third, fourth, and fifth decades of life (peak at 40 years of age) (18, 19). Age under 40 years, significantly increases the risk of developing fistula after perineal abscess (20).

3. Etiology

There are two theories about the cause of anal fistulas: a-congenital cause and b-acquired causes.

A) About the congenital cause, the anal crypts of Morgagni are deeper than normal (1-2 mm) thus, bacteria are trapped much easier and cause inflammation, perineal abscess, and ultimately fistula (21). It is thought that the abnormality of the anal crypts of Morgagni is due to the imbalance between androgen and estrogen, and high androgen level that stimulates sebaceous glands and infection (22).

B) Anal fistulas can be secondary to factors such as persistent perineal pressure (prolonged sitting), inflammatory bowel disease (IBD) (23), or tuberculosis, and immunosuppression (24).

4. Symptoms

Possible symptoms include:

- Pain: It is usually continuous, sharp, and becomes more intense during sitting.
- Itching: Feeling of itching around the anus, which is accompanied by redness and sensation.
- Discharge and bleeding
- Constipation and pain: associated with bowel movement
- Fever.

5. Diagnosis

In most cases, anal fistulas can be identified with the digital rectal exam but in some cases more tests are needed to differentiate it from sexually transmitted infections, IBD, diverticular disease, and rectal cancer. In rare cases, an examination may be performed under anesthesia conditions. Some physicians may use ultrasound, a CT scan, or an MRI for a definitive diagnosis.

6. Treatment

A definitive treatment for anal fistula is surgery, and usually no self-improvement occurs. Depending on the location and type of fistula, various surgeries are adopted:

- Fistulotomy: In 85-95% of cases, this method is used. The whole length of the fistula is cut to allow the surgeon to empty the contents of the fistula and, during one to two months, healing is achieved with a flat scar.
- Seton techniques: This technique is used in cases where the fistula tract passes through the thickness of the anal sphincter, and the cut across the length of the fistula results in cutting the sphincter and consequently fecal incontinence. So in this case, a seton (piece of thread) is placed in the fistula duct.
- Advancement flap procedures: This technique is performed in cases of complex fistulas (with possibility of fecal incontinence). In this technique, during surgery a flap is removed from the rectum with the tissues around the anus, and after removal of the fistula tract it is inserted into the fistula. This method is effective in about 70% of the cases.
- Fibrin glue: This is currently the only non-surgical treatment for anal fistula, in which fibrin glue (liquid form) is injected into the fistula and the fistula is closed. In the short term, it is very effective (about 77%), but in the long run, poor results (14%) are achieved. The benefits of this method include being simple, safe and painless.
- Bioprosthetic plug: This is a new method that, in the short term, is about 80% successful. In this method, a conic shaped plug of human tissue is used to block the internal orifice of the fistula.

- Cell Therapy: One of the most recent non-surgical methods is cell therapy, clinical trials for which are ongoing in the third phase. In this method, stem cells with different sources are used to control inflammation and replace the lost tissue in the fistula tract.

7. Stem Cells in Clinical Trials

In a routine classification, stem cells are divided into two main groups of adult and embryonic stem cells (ESCs). The use of embryonic stem cells in clinical trials is always challenged by ethical problems. Due to easy access from different tissues and a more limited and controlled differentiation, adult stem cells are more suitable candidates for use in medical sciences (25, 26). Mesenchymal stem cells (MSCs) are the most commonly used cells in clinical trials for the following reasons (27):

- Differentiation into various cell types (multi potency): such as bone and muscle (28, 29), cartilage (30), adipocyte (31), hepatocyte (16) and etc.
- Paracrine effect: By secretion of cytokines, chemokines and growth factors, MSCs inhibit apoptosis and proliferate adjacent cells, thereby repairing damaged tissue (32). Another mechanism that stem cell through this inhibit apoptosis in healthy tissue is antioxidant property (32) such as other antioxidant agents (33-39).
- Immunomodulation: MSCs have an immunomodulatory property due to the secretion of suppressor factors of T-cell, B cells, dendritic cells, macrophages, and natural killer cells (38, 39). On the other hand, MSCs do not express MHC, inhibit T cells (through inhibition of NK cells and CD4 and CD8) and create an environment rich in prostaglandins and interleukin 10; thus, after transplantation they will not be rejected (40-42).
- Migration and homing at the site of the lesion: This property is related to cell transport proteins such as chemokines, adhesion molecules, and matrix metalloproteinases (MMPs) (43).

8. Clinical trials of cell therapy in anal fistulas

The first report on the use of stem cells in treatment of anal fistulas is the study of García-Olmo and colleagues in 2003. In this case report, autologous mesenchymal stem cells were extracted from adipose tissue (hADSCs) of Crohn's patient (with recurrent rectovaginal fistula) and used for the treatment of fistula. The results of this study indicated that 3 months after cell transplantation, fistula was closed and no excretion gas or stool from the vagina was seen. Apparently, the cell therapy was effective in this patient, and there was no evidence of ethical or safety problems in the patients (42). After this case report, in 2005, this team developed a Phase I clinical trial that examined the effect of autologous hADSCs transplantation on the repair of anal fistulas in Crohn's disease in 5 patients. In these 5 patients, a total of 9 fistulas were treated with cell therapy and examined weekly for 8 weeks (with 22 months follow up). Finally, 6 fistulas (75%) were lined with epithelium, so they were considered as improved fistulas. In the remaining cases, the external orifice obstruction was not complete (25%) and was associated with secretion, so they were considered as an untreated fistula. This study concluded that the protocols using these cells for treatment of fistulas are feasible and safe, but because of the low sample size, it is not possible to make a definitive statement about the effectiveness of these cells (43). So in 2009, this team conducted a second phase clinical trial with large enough sample size to evaluate the efficacy of hADSCs cells in treatment of complex perineal fistulas. Unlike their first study, which only used cells, in this study, cells were co-administrated with fibrin glue. Perhaps it would have been better to only use the cells in this study to compare the results with the previous study. In this study, 35 patients with complex perineal fistulas associated with Crohn's disease underwent autologous implantation of hADSCs (20 million) with fibrin glue. Improvement of fistula and quality of life were assessed on the eighth week and one year after transplantation. In 17 out of 24 patients (71%) who received the cells co-administration with fibrin glue, fistula recovery was observed. However, fistula recovery was observed in only 4 out of the 25 patients (17%) who received fibrin glue alone. After one year, the rate of recurrence was 17.6%. This team concluded that implantation of these cells together with fibrin glue is safe and effective for treating perineal fistulas associated with Crohn's disease. And administration of cells with fibrin glue is more effective than administration of each, individually, in treatment of this disease (44). In the next study, this team developed a second-phase clinical trial, in which 49 patients were treated with hADSCs (with or without fibrin glue) to further evaluate the effectiveness and safety of autologous implantation of hADSCs. The results of this study indicated the recovery of fistula in 17 out of 25 patients (71%) (hADSCs with fibrin glue) and 4 out of 25 patients (16%) (fibrin glue only) ($P < 0.001$). In patients with suprasphincteric fistula, cell therapy was much more effective than fibrin glue ($P = 0.001$). The quality of life in patients receiving hADSCs was higher than those receiving fibrin glue. After one year, it was found that the rate of recurrence in patients who received hADSCs or fibrin glue was 17.6%. Finally,

the team concluded that injection of 20 to 60 million hADSCs (with or without fibrin glue) was effective in the treatment of complex anal fistulas and, of course, hADSCs were more effective than fibrin glue (45). In phase one and two studies of García-Olmo et al. in 2005 and 2009, the autologous transplantation of hADSCs was used for treatment of anal fistulas (43, 44). Ciccocioppo et al. in 2011, assessed transplantation of bone marrow stem cells (MSCs) (50×10^6) on 10 patients with Crohn's disease who had anal fistula. In this study, patients were evaluated with MRI and endoscopy and followed for a year. Finally, fistula was completely closed in 7 patients, and incompletely closed in 3 patients. Crohn's disease and perianal disease activity indices had significantly decreased in all patients ($p < 0.01$) and mucosal rectum had improved without any adverse effects. Finally, from the results of this study, they concluded that injection of bone marrow MSCs in the anal fistulas associated with Crohn's disease was safe and feasible and could be effective (46). In a following to the phase II clinical trial of García-Olmo et al., Herreros et al. carried out a clinical trial phase III in 2012 and studied the effects of transplantation of autologous hADSCs (20 million) in treatment of complex anal fistulas. In this study, 200 patients with complex anal fistulas were treated with hADSCs (with or without fibrin glue). In one-year follow-up, the results of this study showed that the best recovery rates belonged to hADSCs (57.1%), hADSCs with fibrin glue (52.4%) and fibrin glue (37.3%), respectively ($p = 0.13$) (same in all three groups), and hADSCs (with or without fibrin glue) are safe for use in treatment of complex anal fistulas (45). In 2012, Guadalajara et al. again strongly supported the safety of using hADSCs in treatment of anal fistulas in a study with long follow-up (38.0 and 42.6 months). Also in 2012, Borowski et al. during a study with long term follow-up (2-3-years) applied a combination technique involving the use of mucosal flap and autologous hADSCs transplantation in three patients with complex anal fistulas and showed the efficacy of these cells (47). Lee et al., in a clinical trial phase II in 2013, evaluated the effect of autologous injection of hADSCs (with or without fibrin glue) on 43 patients with an anal fistula associated with Crohn's disease (one-year follow up). The results of the study indicated that in 27/33 patients (82%), the fistula was completely closed without pathologic complications, thus it was concluded that autologous hADSCs transplantation could be an effective therapeutic strategy for Crohn's disease fistulas (48). The tolerability, safety, and potential efficacy of autologous hADSCs in treatment of anal fistulas were confirmed again in 2013 in a clinical trial phase I by Cho et al. who used various doses (1×10^7, 2×10^7 and 4×10^7 cells / ml) (49). 41 out of the 43 patients who participated in Cho et al. study underwent autologous hADSCs transplantation and patients were followed for one year (49). A study with 24-month follow-up indicated that 21 out of 26 patients (80.8%) showed complete recovery of the fistula, and once again concluded that autologous hADSCs transplantation could be an effective therapeutic strategy for anal fistulas (50). In a clinical trial (8 patients with anal fistulas with 72-month follow-up), just like their previous study in 2011 (46), Ciccocioppo et al. showed that autologous bone marrow-derived stem cell transplantation could be a safe and efficient treatment (51). In 2015, Garcia-Olmo D et al. continued their previous studies in 2003, 2005 and 2009 (42-44) and once again emphasized that autologous transplanted hADSCs are effective for treating anal fistulas (52). In studies that have been done so far, autologous stem cell transplantation has been used in treatment of anal fistulas. Now, studies are underway to investigate the transplantation of allogeneic stem cells in treatment of anal fistulas. De La Portilla et al. in 2013, investigated the results of stem cell allogeneic transplantation (with the source of adipose tissue) in the treatment of anal fistulas, for the first time. In this study, 24 patients with anal fistula were treated by injection of 20 million hADSCs in the fistula duct. Patients were followed for 24 weeks after the first dose. In the 6-month follow-up, clinical safty of allogeneic transplantation of hADSCs was confirmed. After a 24-month follow-up, fistulas were completely closed in 56.3% of patients (with hADSCs injection), in 30% of the patients, all of the fistulas (with or without hADSCs injection) were completely closed and 62% of the patients showed draining fistulas. Finally, it was concluded that allogeneic transplantation of hADSCs for treatment of anal fistulas is completely safe. Although these cells are apparently useful for treatment of anal fistulas, further studies are needed to confirm the efficacy of these cells (53). A phase II Clinical trial by Molendijk et al. (two-year follow-up), which was performed on 21 patients with anal fistula caused by Crohn's disease, showed that different doses of allogeneic bone marrow stem cells [(1 × 10 (7), 10 × 10 (7), or 9 × 10 (7)] are all clinically safe, but the best results regarding closing the fistula were observed in 3 × 10 (7) dose (54). Park et al. also investigated the effect of allogeneic transplantation of hADSCs on perineal fistulas associated with Crohn's fistula in a pilot study. Based on the results, Park et al. reported that transplantation of allogeneic stem cells from adipose tissue, like the autologous transplantation of these cells, is safe and effective for treatment of perineal fistulas (55). In 2016, for the second time, a phase III clinical trial of cell therapy for treating anal fistulas associated with Crohn's disease was conducted by Julian Panés and colleagues. In this study, 120 million allogen hADSCs were injected to 107

patients from 49 hospitals for treating anal fistulas associated with Crohn's disease. Finally, the team announced that injection of hADSCs for treatment of anal fistulas associated with Crohn's disease, is a safe and effective treatment in cases that conventional treatments are not effective (56). The most recent studies in the field of cell therapy for anal fistulas are two studies carried out in 2017 that investigated the effect of autologous hADSCs transplantation in treatment of anal fistulas associated with Crohn's disease (57) and not associated with Crohn's disease (58). In 2017, Allan B. Dietz et al., (phase I clinical trial), investigated the effect of autologous hADSCs transplantation with an absorbable matrix (6-month follow-up) on 12 patients with anal fistula associated with Crohn's disease. The results of this study showed that 10 of 12 patients (83%) had complete clinical recovery, and consequently, the safety and efficacy of hADSCs in treatment of anal fistula was once again proven (57). Choi et al., emphasized the safety and efficacy of using hADSCs in treatment of anal fistulas in a phase II clinical trial (50 patients with 6-month follow-up) (58).

9. Conclusion

This review of existing studies for anal fistulas suggests that:

Until now, only two sources of stem cells, in both allogeneic and autologous forms, have been used in clinical trials for treatment of anal fistulas: adipose tissue and bone marrow (mainly adipose tissue).

Except for the two Phase III clinical trials in 2012 and 2016, all clinical trials in this field were conducted in phase one and two with the aim of determining the safety and efficacy of these cells.

Therefore, although the efficacy and safety of cell therapy for treatment of anal fistula have been proven, more studies with different stem cell sources and phase III clinical trials to find the best method to achieve the highest efficacy seem to be necessary.

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11. Conflict of interest

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13. Author contribution

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