Role of Stem Cells in Complex Anal Fistulas

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Perianal fistula

Perianal Fistula is one of the most prevalent anorectal diseases, with an annual incidence of 8 to 10 cases per 100,000 people, affecting more men than women (AMATO, A et al., 2015). Its incidence can reach from 13% to 39% of patients with Crohn's disease, with perianal fistula being one of the common clinical signs of this disease. The American Gastroenterology Association (AGA) classification divides fistulas into simple and complex, taking into account their relationship with the sphincters, number of orifices, presence of complications and involvement of other structures. A detailed physical-proctological examination, complemented by magnetic resonance imaging of the pelvis or two- or three-dimensional transrectal ultrasound, allows for a more accurate determination of the course of the fistulas and their location in relation to the sphincters.
The principles of accepted surgical treatment indicate that fistulas associated with Crohn's disease should always be considered complex.

TREATMENT OF FISTULAS

Complex fistulas, on the contrary, include high transsphincteric with more than 30% of the external and internal anal sphincter involved, suprasphincteric, extrasphincteric, horseshoe, multiple tracts, anterior tract in women, inflammatory bowel disease, radiotherapy, pre-existing incontinence and chronic diarrhea. The approach to complex fistulas is highly challenging due to the high rates of postoperative morbidity and the high rate of recurrence. To date, there is no standard treatment for this condition, although different techniques have advantages, disadvantages and limitations.
Currently, there are minimally invasive techniques that preserve the sphincters and treat the internal orifice and/or fistula tract, such as flap advancement (FLAP), intersphincteric fistula tract ligation (LIFT), clips, fibrin glue (FGS), collagen plug (ACP), collagen paste, laser photothermoablation (FiLaC), video-assisted anal fistula treatment (VAAFT) and platelet-rich plasma (PRP), but none of these methods brought the desired effectiveness. The most radical method, sphincterectomy, poses a risk to the sphincters, leading to incontinence in up to 59% of cases. Sufferers of this type of fistula are generally patients who, after numerous procedures, require protective colostomies or rectal amputation. When it comes to fistula associated with Crohn's disease, it is important to consider treatment combining surgical intervention with the use of monoclonal antibodies associated or not with immunosuppressants.

MESENCHYMAL STEM CELLS (MSCs)

During the embryo development process, multipotent progenitor cells present in the mesodermal layer have the capacity to originate different types of mesenchymal tissues, such as bones, cartilage and muscles. Mesenchymal cells, also known as mesenchymal stem cells or mesenchymal signaling cells, are formed from the differentiation of pericytic, perivascular or mural cells. Studies have shown

that Mesenchymal stem-cells (MSCs) can be found in various vascularized tissues, and are released into the environment when blood vessels rupture and perivascular cells exit.

The origin of MSCs is perivascular, and they have functional abilities, such as immunomodulation and the production and secretion of antibiotic proteins. Additionally, MSCs are identified by cell surface antigens and have the potential to differentiate into multiple types of tissues when cultured "in vitro", which is different from "in vivo" (CAPLAN, A. I., 2011; CAPLAN, A. I., 2017).

TERMINOLOGY FOR MSCS

The use of MSCs derived from adipose tissue (Adipose stem-cells - ASCs) is a new therapeutic approach, which has been proposed by different medical specialties for regenerative function. This name refers to having mesenchymal cells that are collected from fatty tissue, then we continue to call it MSC. MSCs are capable of activating and influencing the tissue microenvironment, stimulating and guiding regenerative activity due to the secretion of mitogenic and immunomodulatory factors. The use of ASCs obtained from the stromal vascular fraction (SVF) has regenerative and immunomodulatory properties that are observed both in vitro and in vivo.

Considering that MSCs will not differentiate into cells that produce regenerating tissues, that is, they will be incorporated and guide the tissues to manufacture, under their guidance, the missing tissue and/or renewing the diseased tissue, the most appropriate name for this cellular niche would be “signaling cells", as it more accurately reflects the fact that these cells are located in places where there is injury or disease and secrete bioactive factors that are immunomodulatory and trophic, facilitating local regenerative processes. What actually happens is that the stimulation triggered by signaling cells joined to the original stem cells of the patient's site and tissue leads the organism towards the construction of a new tissue through bioactive factors secreted by MSCs that were supplied autogenously, leading to increased capacity of natural regeneration of the organism (CAPLAN, A. I., 2017), reaffirming the Hippocratic concept of self-healing.
Due to the cell signaling and guidance capacity, as well as its usefulness in the most diverse medical areas, Caplan cites the need to call MSC Medicinal Signaling Cells, which highlights and emphasizes the ability to direct the regenerative process and not its activity. as a stem cell.
Due to their main characteristics, autologous MSC are the object of great interest from researchers and surgeons from different areas of medicine, including more recently proctology, as a new therapeutic approach, as their grafting at the surgical site has proven to be a valid, safe and effective in regenerative treatments.

The use of MSCs is a real advance for the treatment of complex rectal fistulas, with or without a prior surgical approach (CHOI et al., 2019) and, although its use in the therapy of complex fistulas is in its initial phase, excellent results have already been achieved. observed in practice. MSCs promote the stimulation and conduction of differentiation of a wide variety of local cells and tissues and their ability to secrete growth and anti-inflammatory factors represent a fundamental therapeutic action for the treatment of complex anal fistulas.

Surgical treatment of Complex Rectal Fistulas using Adipose Mesenchymal Stem-Cells

The application of adipose stroma containing stem cells, autologously, in just one surgical procedure is a method that aims to administer MSCs, contained in the adipose tissue in CRF. Although the procedure has been performed for several years, only recently has a more uniform surgical protocol been established. The exact location and classification of the fistula and the collection site of standardized material to a depth of 15 mm are of fundamental importance before the planned

surgical procedure, therefore, it is necessary to carry out a detailed physical examination, associated with complementary exams and the correct collection of the material.

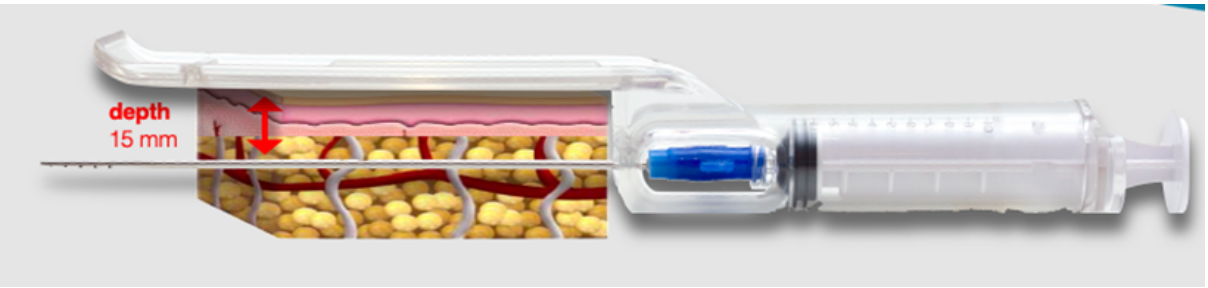
Procedure

A new, standardized technique was developed aiming to obtain adipose tissue with an intact stromal vascular fraction (SVF) and MSCs with high regenerative capacity. This technology is used to collect, process and inject refined adipose tissue, characterized by easy handling capacity and great regenerative potential, based in MSCs derived from hypodermic adipose tissue up to 15 mm deep.
In this method, adipose tissue is collected in small dimensions of the adipose cell clusters of about 0.2 to 0.8 mm. In the collected tissue , pericytes are retained within an intact stromal vascular fraction and are ready to interact with the recipient tissue after homologous transplantation, as signallers, initiating the regenerative process.
This method has been used throughout the world in plastic surgery, orthopedics and others, with cell collection following a common pattern, changing only according to the proposed surgical procedure, with promising results and no major inconveniences. The technology exists as an intra-operative system to obtain an ideal final product that can be used immediately.
It is a way of treating the tissue delicately using the individual's own hypodermic adipose tissue to stimulate the regeneration of areas that have suffered damage. Local administration of adipose tissue containing MSCs allows the healing of more than 57% of complex fistulas, including the most difficult cases of patients who have undergone repeated treatments without satisfactory outcome.

COLLECTION OF MSCs

The hypodermic tissue collection system is designed to minimize the risk of inserting the cannula too deeply and ensure that the tissue is collected in the correct, standardized plane. Adipose tissue is harvested in superficial layers, up to 15mm deep (Fig. 53.7.1), as these layers are richer in stromal vascular fractions (SVF) and signaling cells, which have an average yield of 150,000 to 250,000 SVF cells/ m during collection, reaching 3,000,000 in 72 hours. The smaller the clusters of adipose tissue, the smaller the size of the clusters and the more fluid the tissue will be.

Figure 53.7.1 - Device used to remove tissue, 15mm from the skin.

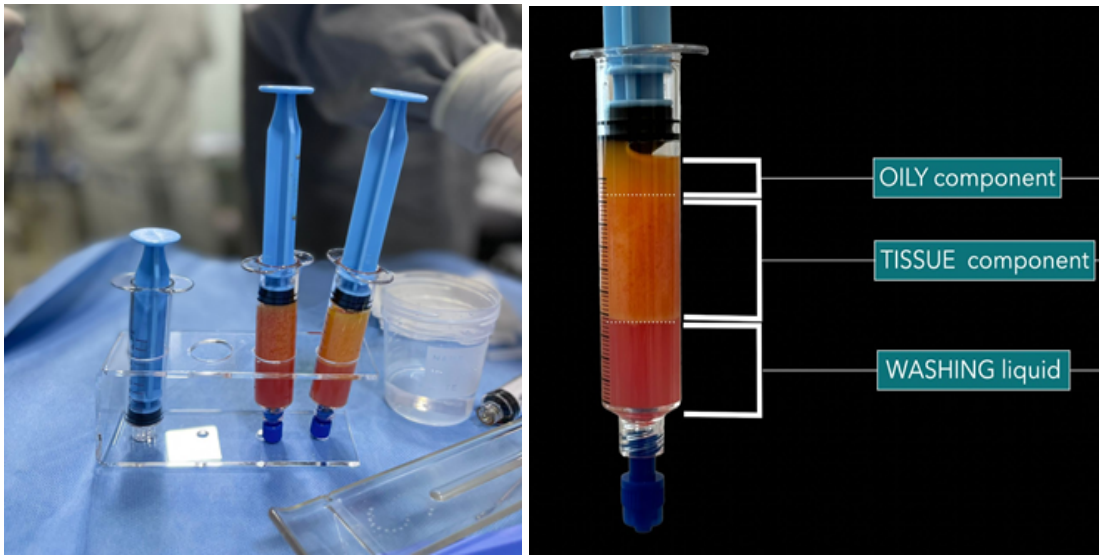


Fonte: Curso básico A. Gennai

For collection of donor site adipose tissue, the lower abdomen or thigh (trochanteric region) are the preferred sites. Before collection, a modified Klein solution, consisting of 20 ml of saline solution, 10 ml of 2% lidocaine and 0.3 ml of adrenaline, is injected into the anterior abdominal wall or trochanteric region, using a specific disposable cannula. After waiting 10 minutes, without the need for centrifugation or cell culture, the hypodermic tissue is collected through the special cannula, with side port holes that collect smaller sizes of adipose tissue clusters 800 microns. Wait 10 minutes and this cannula (the same one used to inject the anesthetic solution) allows the collection of superficial fluid tissue in a standardized, easy, effective and safe way. The special cannula is connected to a Vaclok® syringe and the aspirated tissue is collected in 5 ml fractions in 10 ml syringes.

Saline solution (S.F.0.9%) 5ml per syringe is then added (filling the entire syringe) and the content is transferred to another syringe. The entire process is carried out in a closed system and in saline solution, minimizing any cellular damage. The adipose tissue resulting from the collection and the saline solution is positioned to decant and the excess saline solution is discarded (Fig. 53.7.2). Then, the product is transferred to another syringe, from one syringe to another, several times, connecting the two with a transfer, to remove oily substances and blood residues that have pro-inflammatory properties.

Figure 53.7.2 - Decantation and after decantation, the removed tissue is divided into 3 parts, where we apply only the central part.



Source: Photos by the author and Gennai A.

LOCAL FISTULA TREATMENT

The surgical procedure for complex fistulas, involving the local administration of MSCs, is performed under general or local spinal anesthesia with the patient in the gynecological or lithotomy position, which is our preferred position. It is a minimally invasive, sphincter-sparing procedure that is performed in a single surgical step.

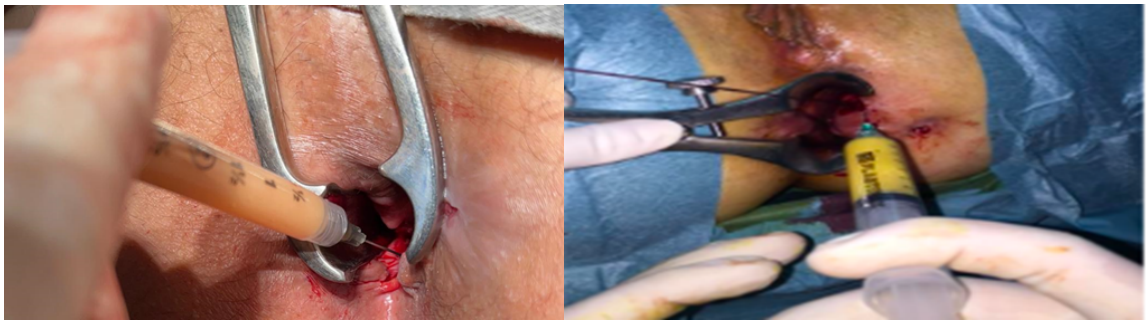
The first and fundamental step involves curettage or cleaning with a "fistula-brush" or careful curettage in all paths from the fistula to the internal orifice, in order to remove the epithelial lining, inflammatory tissue and necrotic tissues. The appearance of bloody secretion along the path indicates that there was adequate cleaning. This stage ends with abundant washing of all fistulous tracts with saline solution.

Closing the internal orifice is the second fundamental part of the procedure, which is carried out through the construction of a mini flap where the muscles of the internal orifice are first closed with simple sutures made of 2/0 polydioxanone and the mucous layer is closed with Vicryl 2 /0 and covered by the mucous and submucosal layer of the mini flap created, and sutured with simple, firm stitches, facilitating the third stage of the surgical procedure, which is the local administration of signaling cells.

The solution containing autologous signaling cells is injected into the muscularis, where the internal orifice was closed (4ml) and into the submucosa of the flap next to the internal orifice (4ml), and along the entire length of the fistula 1ml for each cm of fistula, (Fig. 53.7.3) It is not necessary to

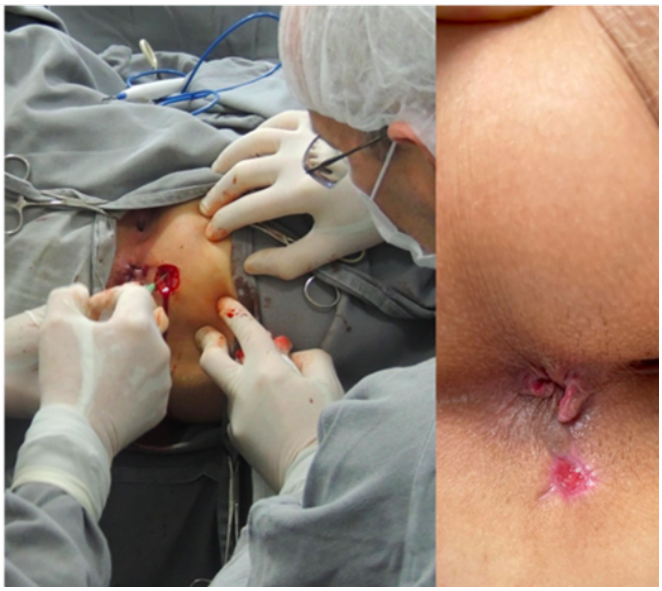
inject into the fistulous tract so the external orifice is enlarged and left open (Fig. 53.7.4). The procedure ends with the placement of a loose dressing without leaving Seton and without the administration of disinfectants or other agents that may be toxic to signaling cells (hydrogen peroxide is particularly contraindicated). The standardization of the signaling cell administration technique at the periphery of the pathways and the determination of optimal doses and injection sites are the crucial points that we determined in our studies to maximize the therapeutic effect in the treatment of complex perianal disease.

Figura 53.7.3 - Application of AMSCs in the internal orifice (muscle), in the flap and along the fistula.



Source: Photos by the author.

Figura 53.7.4 - Right: Enlargement of the external orifice of the fistula, with the application of AMSCs. Left: External orifice occluded 1 month after the procedure.



Fonte: *Fotos do próprio autor.*

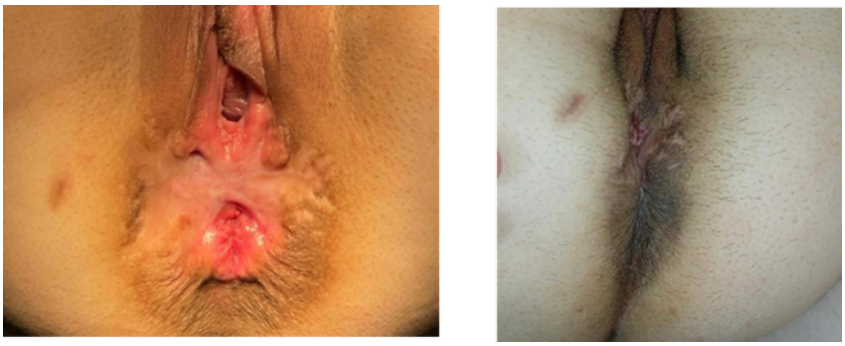
In this technique, adipose tissue is gently collected into smaller clusters and washed in saline solution, freeing it from pro-inflammatory oils and blood residues.

The good result of this technique also applies to the double-layer closure of the internal orifice, with a small flap that prevents the continuous passage of fecal residue through the fistula, resulting in an excluded fistulous tract in which the inflammatory tissue already removed by "fistula -brush" or curettage and the external orifice enlarged and left open allowing discharge and ensuring closure of the internal orifice to the external. Liquid hypodermic adipose tissue injected first into the muscles near the closed internal orifice and then into the edge of the small flap in the layers around the internal orifice and then around the fistula path will speed up. Hypodermic adipose tissue is not injected into the fistula tract because it does not have any volume activity, as MSCs only act as

immunomodulatory agents to stimulate tissue regeneration, supporting the natural healing process, complications are minimal and easily resolved.

There are promising results with a cure rate of 83.3% in patients without previous treatment, as shown in (Fig. 53.7.5). It is believed that due to the abundance of fibrotic tissue in the healing process of chronic fistulas, the number of recurrences is reduced by the use of MSCs, through regenerative stimulation.

Figura 53.7.5 - On the left, preoperative fistula, showing an abundance of fibrous tissue in a fistula with multiple anterior approaches. On the right, fistula in the late postoperative period, after application of adipose tissue with MSC.



Fonte: *Fotos do próprio autor.*

STUDIES

Meta-analysis

Surgical treatment of Complex Rectal Fistulas is still a challenge, even for a surgeon with good practice. However, the surgical method is the best option, aiming to improve quality of life and control possible future complications.

The principle of surgery is to preserve the sphincters and prevent recurrence, which are often not achieved in current surgeries. Therefore, stem cells emerged as a treatment, seeking to achieve the two purposes of the surgical approach to complex fistulas, and due to their ease of obtaining and their immunomodulatory capacity, they are widely used today.
A 2018 meta-analysis by Choi et al. evaluated 16 articles published between 2005 and 2017 for the treatment of Complex Rectal Fistulas. Only recurrent fistula cases were included, which could not be addressed by conventional means. The study achieved an overall success rate of 62.8% in closing the fistula, showing a benefit in the use of stem cell therapy.

Research with autologous and allogeneic cells, when compared separately, autologous cells have a higher rate of fistula closure. In randomized clinical trial studies, the group receiving stem cell therapy achieved better healing compared to the control group (OR: 0.379, 95%, CI 0.152 - 0.947). Furthermore, in articles where the number of injected cells was adapted to the size and characteristics of the fistula, there was a higher success rate in closing the fistulas.

Currently, several articles, including phase 3 clinical studies (ADMIRE-CD and INSPECT), have been carried out for the treatment of Complex Rectal Fistulas using MSC. Darvadstrocel, an allogeneic MSC-expanded cell suspension. It was the first advanced therapy using MSCs approved by the Federal Drug Administration (FDA). It currently has a benefit in cases of fistulas in Crohn's disease and fistulas of cryptoglandular origin.

The use of autologous MSC cells is a therapy with therapeutic outcomes similar to the allogeneic technique, and with the benefit of being harvested and engrafted to the same individual, injected in the same surgical time, having a lower cost and being easily collected. Using the keywords (complex rectal fistulas) AND (adipose stem cells) in Pubmed, with a filter for the last 5 years, we obtained 27 articles, of which: 1 meta-analysis (already cited), 8 clinical trials and 6 randomized clinical trials. This is a current topic, with promising results in patients with Complex Rectal Fistulas, and which in most cases is able to act by preserving the sphincters and promoting the closure of the fistula path.

FINAL CONSIDERATIONS

Despite all the therapeutic potential of homologous adult stem cells, there is much to be explored. Literature analysis and clinical observation show that their use has not yet reached its full potential. From a surgical point of view, one of the main challenges when using it in the perianal region for fistula treatment is the fluid consistency of the preparation used for administration. Depending on the methodology used, it can lead to loss of biological material during preparation and injection, compromising the effectiveness of the procedure.

The method to be used must reduce the size of the collected clusters, and without centrifugation or enzymatic reaction, prepare the material only by washing it in saline solution (S.F.0.9%). This method has proved quite promising as it reduces the loss of biological material during the washing of the inflammatory content from the harvested tissue, resulting in a greater number of engrafted cells, thus improving the effectiveness of the treatment.

It is important to remember that mesenchymal cells (MSCs) have sufficient structural support to allow their survival during the first few days before being overtaken by newly formed vessels and being able to demonstrate their full therapeutic potential. Therefore, it is critical to continue developing new strategies to improve the survival and efficacy of MSCs in different clinical contexts in order to reach their full potential. There is a high concentration of regenerative mesenchymal cells in hypodermic adipose tissue, where we collected the material, in 1g of fat tissue 5,000 mesenchymal cells can be isolated (500 times higher than the equivalent in bone marrow) so in 4ml, around 20,000 cells and in 15ml about 360,000 cells, therefore abundant regenerative cells.

Although there are still many questions to be answered, the use of mesenchymal stem cells is one of the most promising therapeutic options for this condition, and research is underway to answer these questions.

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