

# Mesenchymal stem cells in treatment of perianal and rectovaginal fistulas

## Abstract

Perianal fistulas are still a major challenge in everyday clinical practice. Particularly difficult fistulas, like those in Crohn's Disease, often require multiple aggressive surgical interventions, with high risk of sphincter damage and incontinence. Intensive trials have been conducted for over 10 years on the treatment of difficult fistulas with mesenchymal stem cells (MSC). This paper presents a short review of basic mechanisms of mesenchymal stem cells in healing of damaged tissues and results of major clinical trials. MSC in perianal fistulas, regardless of research protocol has a 70% healing rate in recurrent fistulas and in fistulas in Crohn's Disease. Further research is needed to precisely define the indications for MSC-based treatment of tissue damage, and particularly perianal and recto-vaginal fistulas.

**Keywords:** fistula-in-ano, recurrent fistula, complex fistula, crohn disease, adipose-derived mesenchymal stem cells, clinical trials

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**Abbreviations:** ASC, Adipose-derived mesenchymal stem cells; MSC isolated from adipose tissue; bFGF, basic fibroblast growth factor; MSC, mesenchymal stem cell; HGF: hepatocyte growth factor; ILGF-1, insulin-like growth factor 1; VEGF, vascular-endothelial growth factor

## Introduction

Perianal and rectovaginal fistulas are pathological connections between the rectum and adjacent structures in the rectoanal region. About 80% of perianal fistulas are of cryptoglandular origin the remaining 20% develop in Crohn's Disease, after trauma, or have iatrogenic etiology, etc.

Standard in perianal fistula treatment are cutting (fistulotomy, fistulectomy) or non-cutting (seton) surgical procedures, to remove granulation tissue, necrosis and translocated mucosal cells. The success rate of primary surgical treatment of perianal fistulas reaches about 70%.<sup>1</sup> In the remaining cases, there is no healing of the fistula after the procedure, or the fistula reoccurs after initial healing. Fistula recurrence rate in Crohn's Disease is relatively high (34%)<sup>2</sup> which is due to constant inflammation of the intestines and soft tissues. Therefore surgical approach is preceded by immunosuppressive treatment, including anti-TNF $\alpha$ , which is necessary for proper soft tissues healing.

Regardless of the fistula etiology, the disease greatly reduces the patients' quality of life both due to recurrence and incontinence.<sup>3</sup> The metaanalysis by Blumetti et al.<sup>4</sup> shows, that aggressive surgical approach (eg. fistulectomy) has a greater chance of successful healing, at the same time increasing the risk of fecal incontinence. Therefore, there is a constant search for better alternatives for fistula treatment.

In 2003 Prof. Garcia-Olmo made the first attempt to treat a rectovaginal fistula in a 33-year old female patient with Crohn's Disease, using the mesenchymal stem cells derived from the fatty tissue of the patient (Adipose-derived mesenchymal Stem Cells).<sup>5</sup> By implantation of about 9mln of autologous stem cells he achieved complete closure and lack of recurrence of the fistula. From that

time, many approaches to soft tissue treatment with MSC were made. This paper presents a short review of clinical research on the MSC treatment of perianal and rectovaginal fistulas so far.

## Discussion

### Mechanism of action

Mesenchymal stem cells exist in the perivascular niche of almost all tissues,<sup>6</sup> however, the most useful source of these cells are bone marrow, adipose tissue and umbilical cord.<sup>7</sup> In classic approach, MSC are pluripotent cells that support the natural healing process by increasing the overall pool of cells (self-renew) and differentiation in the direction of mesenchymal cells (fibroblasts producing collagen, adipocytes, myocytes, osteocytes, etc.).<sup>8</sup> In addition, MSC modulate the healing process by stimulating dermal fibroblasts to divide, migrate toward the injury, and the expression of collagens.<sup>9</sup> In hypoxic conditions which occur on the edges of tissue damage, MSC secrete growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), insulin-like growth factor, and other<sup>10</sup> therefore inducing revascularisation.<sup>11</sup> Mesenchymal stem cells also possess immunosuppressive properties.<sup>12,13</sup> In a mouse model of inflammatory bowel disease MSC suppress Th1 lymphocytes, which leads to a reduction in clinical and histological symptoms of inflammatory bowel disease.<sup>13</sup> Multifactorial mechanism of action of MSC responds to the need for proper healing of soft tissue, but we still do not know which of the effects described by basic science have clinical significance. What's more, there are no direct studies on the characteristic of the microenvironment of the damaged tissue, and in particular its "weak spots" in relation to the properties of the MSC, which could allow the development of better treatment strategies.

### Clinical outputs

Since 2005 at least 9 clinical trials with MSC treatment of the perianal fistula were conducted.<sup>14-24</sup> The summary of prospective studies are shown in Table 1. In all studies, 225 cell-treated patients were completed the protocol, 107 were healed in 8-24 weeks of follow-

up. In all trial administration of the cells was combined with surgical closure of the fistula (one-stage procedure). Patients received 9 to 139 million cells suspended in fibrin glue<sup>15,16,23</sup> or in saline solution.<sup>15,19-22</sup> The cells were administered into the walls of the fistula and into its lumen.

N: number of cell-treated patients who completed the protocol, H – number of cell-treated patients with healed fistula in 8-24 weeks follow-up; \* - Number of non-Crohn/Crohn patients; \*\* - Compassionate-use programme.

It is difficult to determine the efficacy of MSC / ASC as each of these studies had a slightly different protocol. For this reason, the total numbers of patients were counted - patients who received mesenchymal stem cells (isolated from bone marrow and adipose tissue, auto and allogeneic, alone or with fibrin glue) in clinical trials or registered treatment programs (compassionate use). Only prospective studies were taken into account, and the endpoint (ie.

“healed”) was considered as clinical healing in the period from 8 to 24 weeks after administration of the cells (the status of “partially healed” was classified as “not healed” unless the healing was achieved up to 12 months after the cells were administered).

Among 225 (or 154) patients who received the MSC or ASC status of “healed” were reported in 138 (54%) (or 103 (67%) - excluding non-pioneering center from the largest clinical trial conducted by Herreros et al.<sup>15</sup>) cases. The healing rate in non-Crohn fistulas were 45% (n=148 cell-treated patients) and (or 68% (n=47) excluding non-pioneering centers from Herreros et al.). We decided to separately present a results from Herreros et al. because of only in the pioneering center outcomes of cell-treatment patients were significantly better than in the rest centers (the healing rate in 19 centers were 50% (n=124) but in pioneering center reached 70% (n=23)). This fact suggest major influence of experience in cell-based products management on the final results.

**Table 1** Summary of clinical trials and compassionate-use programmes with mesenchymal stem cells therapy in treatment of fistulas

	Published clinical trial	Etiology of fistula	Type and amount of cells	N / n	H / h
1	Garcia-Olmo et al. <sup>14</sup>	Crohn	Autogenic ASC, 3-30 mln	9	6
2	Garcia-Olmo et al. <sup>23</sup>	both	Autogenic ASC, 20 mln	17/ 7*	12/ 5*
3	Ciccocioppo et al. <sup>18</sup>	Crohn	Autogenic MSC, 54-139 mln	10	7
4	de la Portilla et al. <sup>20</sup>	Crohn	Allogenic ASC, 20-40 mln	16	9
5	Herreros et al. <sup>15</sup>	non-Crohn	Autogenic ASC, 20 mln	124	51
		Results in multicenter trial excluded a pioneering center		101	35
		Results in the pioneering center (Garcia-Olmo)		23	16
6	Lee et al. <sup>16</sup>	Crohn	Autogenic ASC, 9-42 mln	33	27
7	Cho et al. <sup>17</sup>	Crohn	Autogenic ASC, 30-40 mln	9	3
8	Garcia-Olmo et al. <sup>22**</sup>	Both	Auto- autogenic ASC, no data	7 / 3*	4 / 2*
9	Garcia-Arranz et al. <sup>24</sup>	Crohn	Allogenic ASC, 20-40 mln	5	3

In the case of Crohn's Disease overall healing rate was 66% (n=107) which include Lee et al.<sup>16</sup> where reported healing rates even up to 82%. There are several different strategies reported in the literature (use of auto or allogenic cells, the administration of from 9 to 139 million cells, the administration of cells in fibrin glue, or alone, administration of cells at once or sequentially until healed, and various ways to provide the fistula before administration of the cells). Therefore it is very difficult to compare results between studies. However, achieved results show, that there is a positive effect of the MSC on soft tissue healing.

In the presented studies some of AE (adverse effects) were reported but only in Herreros et al.<sup>15</sup> there were reporting in systematic condition. The most frequent adverse effects.<sup>15</sup> were a proctalgia (43%) (that was also reported by our team (data not shown)) and abscess drainage (22.4%). Pain, perianal abscess, pyrexia, swelling and pruritus were also reported in around 10% or less patients who were inclu.<sup>15</sup> Frequency of these effects were no statistically differences within groups which received the cells or fibrin glue. In longer observation the most probability AE, directly associated with cell transplantation - uncontrolled cellular growth were no reported in Herreros et al. and another publication as well.

Lack of differences between groups in occurring of the AE and lack of the uncontrolled cellular growth suggest that reported

adverse effects were more related to the act of administration an ascetical products risky perianal location than the products were a mesenchymal stem cells. It must be stressed here that cells cannot be sterilized as typical pharmaceuticals (ex. gamma sterilization) even more a precise bacteriological status of cell-living product is unknown at the time of cells administration (all methods of microbiological assessment have to be incubated at least a couple of hours to observe a pre-result; but an aseptic examination that have to be conduct to precise assess of the final product require a 2 weeks of incubation (accordingly with European Pharmacopeia). From that reasons using of cell-living products demand a high quality of cell production in cell banks, trained medical staff as well as perfect coordination between themselves.

## Conclusion

In clinical trials so far, the healing rates of MSC fistula treatment was about 70%. Pleiotropic effect of action of the stem cells seems to be particularly beneficial in the process of perianal fistula healing, but we still don't know, whether the cells are permanently built into the tissue, and what clinical value have the properties of the cells we described. From that reason there is still grate necessity to explore a mechanism of soft tissue healing driven by autogenic as well as allogenic MSC. Developing of spin-labelling technics and magnetic resonance imaging seems to be useful tools in investigation of

“fate of cells” after implantation and can be directly implemented in clinical trials protocols. The explanation of these aspects will help to develop better strategies to introduce the cells and thus contribute to the definition of precise indications for such treatment.

The cell-based therapies that are still in investigational phase require from all scientific and medical society to treat the patients only in restricted conditions of registered clinical trials or compassionate use programs to decrease chance of serious adverse effects. In over 10-year long research on safety and effectiveness of the mesenchymal stem cells in treatment of soft tissues give hope for a new, safer therapeutic option, especially useful in difficult fistula cases but still need more effort to better understand a nature and consequences of cell-based products.

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## Conflicts of interest

The authors declare no conflicts of interest.

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