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**Stem cell therapy for faecal incontinence: Current state and future perspectives**

Trébol J *et al*. Stem cell therapy for faecal incontinence

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# Abstract

Faecal continence is a complex function involving different organs and systems. Faecal incontinence is a common disorder with different pathogeneses, disabling consequences and high repercussions on quality of life. Current management modalities are not ideal and the development of new treatments is needed. Since 2008, stem cell therapies have been proven, 36 publications have appeared (29 in preclinical models and 7 in clinical settings) and 6 registered clinical trials are ongoing. Some publications have combined stem cells with bioengineering technologies. The aim of this review is to identify and summarise the existing published knowledge of stem cell utilization as a treatment for faecal incontinence. A narrative or descriptive review is presented. Preclinical studies have demonstrated that cellular therapy, mainly in the form of local injections of muscle-derived (muscle derived stem cells or myoblasts derived from them) or mesenchymal (bone-marrow or adipose derived) stem cells, is safe, stimulates the repair of both acute and subacute anal sphincter injuries and obtains some encouraging functional results. Stem cells with normal cells on bioengineered scaffolds have achieved the successful creation and implantation of intrinsically innervated anal sphincter constructs. The clinical evidence, based on adipose-derived stem cells and myoblasts, is extremely limited but has yielded some promising results, and appears to be safe. Further investigation in both animal models and clinical settings is indispensable to drawing conclusions, but if the preliminary results are confirmed, stem cell therapy for faecal incontinence may well become a clinical reality in the near future.

**Key words:** Faecal incontinence; Anal sphincter; Cell implantation; Cell therapy; Stem cells; Tissue engineering

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**Core tip:** Faecal incontinence is very frequent and associates severe consequences for patients. Available treatment outcomes are not as good as they should be, mainly in the long-term. Stem cells, combined or not with bioengineering, could improve these results, as demonstrated in other clinical settings. We performed a descriptive review of the published literature about faecal incontinence and stem cells and discuss the existing limitations and concerns. Preclinical studies have confirmed the feasibility and safety and show some interesting results; the limited clinical experience confirms the safety and potential efficacy. However, further studies are needed to obtain clear conclusions.

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# INTRODUCTION

Faecal incontinence (FI) is a highly prevalent nonfatal illness associated with considerable embarrassment, anxiety and poor quality of life. In a systematic review by McMillan, it was estimated to occur in 11%-15% of adults[[1](#_ENREF_1)] and Nelson found it in 2.2% of the general population and 47% of institutionalised individuals[[2](#_ENREF_2)]. It is difficult to know the real prevalence due to its psychosocial repercussions, as patients tend not to report it to their physicians and physicians rarely ask about it.

The physical repercussions are limited, but psychosocial ones are devastating: loss of self-confidence, disability, body image alteration, social isolation, anxiety, depression, etc. A British study observed a four-fold increase in anxiety and five-fold increase in depression, with a significant association between both and faecal incontinence[[3](#_ENREF_3)]. Furthermore, it could also lead to job losses and is the second highest cause of institutionalisation. Studies focusing on quality of life reflect significant repercussions in multiple components, such as physical[[4](#_ENREF_4),[5](#_ENREF_5)] and mental[[5](#_ENREF_5)]; generally, the greater the incontinence, the greater the deterioration it causes (*P* < 0.01)[[5](#_ENREF_5)]. A Spanish study showed an independent association with quality of life and mental health worsening (OR 2.088 and *P* = 0.017)[[6](#_ENREF_6)].

The economic impact is high and very difficult to estimate, but it consists of direct (diagnostic test, treatments, care, *etc*.) and indirect (job production, secondary treatments, such as for psychological consequences, e*tc.*) costs. Indirect costs are harder to calculate and account for more than half[[7](#_ENREF_7)]. In a Seattle study, annual healthcare costs were augmented significantly in multivariate analysis up to 2897$ in 2005 (pads, barriers or institutionalisation not included)[[4](#_ENREF_4)]. In a Dutch study, global costs grew by 2169€ yearly for each patient[[7](#_ENREF_7)].

The prevalence is higher among women, the elderly, people with poor health or physical limitations, and those residing in nursing homes. Other risk factors include pelvic radiation, pregnancy, pelvic injury associated with vaginal delivery, anorectal surgery, diarrhoea, faecal impaction, some neurological conditions and diabetes.

Bowel continence is a very developed function depending on complex sensory and motor interactions between the rectum, anus, external anal sphincter (EAS), internal anal sphincter (IAS), puborectal muscle and their vascularisation and innervation. When one or more of these structures or interactions are disrupted to such a degree that the others are unable to compensate, incontinence appears. Therefore, FI is a multifactorial disease. The most frequent morphological alteration found is a sphincter lesion in almost 60% of patients, with most of them being obstetric (30%-40%). Sphincter lesions during delivery range from 11%[[8](#_ENREF_8)] to 26.9%[[9](#_ENREF_9)], increase with every pregnancy, and cause incontinence in 76.8%-82.8% of patients[[9](#_ENREF_9)].

Although sacral neuromodulation has been growing exponentially in recent years, surgery remains the treatment of choice for the most severe or refractory cases, mainly when sphincter lesions are present. There are a lot of surgical techniques, but sphincter repair is the most successful for sphincter injuries. Sphincter repair has shown good results in the short-term: excellent-good in 66%, moderate in 22% and poor in 12% of patients[[10](#_ENREF_10)]. However, these outcomes do not persist in the long-term; Halverson and Hull found that 54% were fully incontinent and only 14% were fully continent 69 mo later[[11](#_ENREF_11)]. Similarly, the review by Glasgow and Lowry, with 16 publications comprising almost 900 reparations, observed an almost constant decline in initially subjective “good” outcomes in the long-term. Despite these worsening results over time, most patients remained satisfied (also in quality of life). No failure predictive factors were found[[12](#_ENREF_12)]. The reasons for this decay are not well understood.

Stem cell (SC) therapy has been demonstrated to be safe and to obtain promising results in a wide variety of clinical and experimental settings: Haematological, cardiovascular, neurological, digestive, traumatic, endocrine, renal and metabolic conditions are some examples. The most commonly used are haematopoietic stem cells (HSCs)[[13](#_ENREF_13)], mesenchymal stem cells (MSCs)[[14-16](#_ENREF_14)] or adipose-derived stem cells (ASCs)[[17-19](#_ENREF_17)]. For example, ASCs have been tried in environments that are particularly unfavourable for wound healing, such as experimental colitis[[20](#_ENREF_20)], sepsis[[21](#_ENREF_21)], anal fistula[[22-29](#_ENREF_22)], Crohn´s patients[[30](#_ENREF_30)] and experimental colonic[[31](#_ENREF_31),[32](#_ENREF_32)] and tracheal[[33](#_ENREF_33)] anastomoses with favourable outcomes.

Based on the published literature, on our group’s experience using ASCs in experimental and clinical settings (conducting or participating in more than 6 clinical trials with autologous or allogeneic ASCs) and with FI treatment, our aim was to review published literature related to FI and stem cell therapy and currently ongoing clinical trials. To the best of our knowledge, there is only one review on this field from Gräs *et al*[[34](#_ENREF_34)], including also tissue engineering studies published prior to June 2015.

**SEARCH**

We performed an exhaustive search of the published literature in the United States National Library of Medicine database (“PubMed”), using the following terms: “faecal incontinence”, “anal incontinence”, “stem cells”, “progenitor cells”, “cellular therapy” and “cell therapy”. Only studies published in indexed peer-reviewed journals were selected. “Similar articles” in PubMed and references of the selected studies were also analysed to detect potentially includable articles. Related to bioengineering, only publications combining it with SCs were considered for this review. The United States National Library of Medicine official registry of clinical trials “ClinicalTrials.gov” ([www.clinicaltrials.gov](file:///Users/lima/Desktop/2018-06-29_New_Journals_Send_to_Ma_L/39955/www.clinicaltrials.gov)) and the EU Clinical Trials Register ([www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu)) were searched using the same terms to detect ongoing registered clinical trials. Both searches were performed on 1st April 2018.

The high variability on FI models, cellular products employed and the methodology of applying it or evaluating their results, make it impossible to perform a meta-analysis. Therefore, a narrative or descriptive review is presented.

**STEM CELLS APPLIED FOR FAECAL INCONTINENCE; A BRIEF OVERVIEW:**

The pioneering report in this field was from Lorenzi *et al*[[35](#_ENREF_35)] in 2008 in an animal model. From that point onwards, several articles have been published, mostly using animal models. However, since 2010, a few experiences with humans have gradually appeared.

We have identified a total of 36 publications eligible for a deeper analysis. Twenty-nine are preclinical studies on animal models, some combining SCs with bioengineering strategies, trying to create a biocompatible and implantable EAS or IAS construct. Seven publications are on humans. Also, 6 registered clinical trials were found which are “active” or apparently ongoing. In the following sections, we are going to analyse and summarise the publications, ordering them using the internet publication date or “Epub” date.

**ANIMAL STUDIES PUBLISHED**

In the 29 selected preclinical animal studies, high heterogeneity on employed animals, faecal incontinence models, type of repair, kind of SC applied and response evaluation system were applied. Overviews of the following aspects are presented in Tables 1-3: injury model and repair (Table 1), kind of SC employed (Table 2) and bioengineering strategies combined with SCs (Table 3). Type of animal used and employed adjuvants to SCs is mentioned later.

The first publication was by Lorenzi *et al*[[35](#_ENREF_35)] in 2008. They performed a left lateral selective sphincterotomy without specifying its length in male rats. The authors divided the animals into four groups of 8. Two received sphincter-injected BM-MSCs after non-overlapping repair (autologous: group C or allogeneic associated with immunosuppressive drugs: group D) and were compared with groups of sham injury and saline injection (A) and injury, repair and saline injection (B). There were no relevant complications or exitus. After 30 d in histologic examination, a significant decrease in muscle tissue was observed at the site of repair, but morphometric analysis in groups C and D revealed a significantly greater muscle area than in group B (*P* < 0.05), but significantly lower than in group A. Infunctional assays, with *in vitro* contractility, a significantly better response to electrical stimulation and relaxing capability appeared in groups C and D compared with B (*P* < 0.05). No significant differences were found between groups C and D.

In the same year, Kang *et al*[[36](#_ENREF_36)] published an investigation using cryoinjury in Sprague-Dawley female rats, without specifying the damaged volume (although the probe is applied against the right sphincter hemisphere). The authors studied injection with microscopic guidance of 3 × 106 autologous muscle-derived stem cells (MDSCs) into the sphincter damaged zone. Fifteen rats were divided into three groups: control (A); cryoinjury (B); and cryoinjury and cell therapy (C). Evaluations were performed one week after the injury. On muscle strip *in vitro contractility assays,* cryoinjury significantly decreased contractility and MDSCs increased its amplitude without reaching statistical significance. On histological examination, they found labelled cells in all animals at the MDSC injection site, confirming survival and tolerability (there were no immune response), and also differentiated muscle masses with variable orientations, suggesting partial myofibre (smooth and skeletal muscle) regeneration.

In 2009, Saihara *et al*[[37](#_ENREF_37)] evaluated the evolution of allogeneic myoblasts from female F344 rats (at 1-4 wk) implanted into nude mice on subcutaneous tissue, damaged thigh muscles and healthy levator ani. Myoblasts were most efficiently obtained from more juvenile rats. SCs were capable of forming myotubes *in vitro* and on subcutaneous fat at 3 wk and became integrated into damaged muscles with myofibre formation at 4 wk. Nevertheless, in healthy muscle, myoblasts survive in smaller numbers, surround muscle without integrating into it and form myotubes but not myofibres. Therefore, injury stimulus may be fundamental to myofibre formation.

Aghaee-Afshar *et al*[[38](#_ENREF_38)] published the first rabbit model in the same year, applying a surgical damage (EAS lateral sectioning) without repair. Two weeks later, 7 animals per group received human umbilical cord stem cells (uSCs, 104), allogeneic rabbit BM-MSCs (104), culture medium or saline solution. These groups were also compared with 3 non-injured animals, all of which were evaluated before damage, before treatment and two weeks later. Clinical results: Complete sphincter competence was found in 4 out of 7 patients with BM-MSCs compared with 2 out of 7 with uSCs, and partial competence in 2 out of 7 with culture medium. On the electromyograph, there was a significant decrease on peaks per second after the injury and a significant increase with BM-MSCs compared with pre-treatment values and controls; a non-significant increase appeared with uSCs and no increase appeared in other groups. Both kinds of SCs are able to survive at the injury site. Histopathologic evaluation showed a normal or muscle-dominant sphincter structure in all animals receiving BM-MSCs and a fibrous-dominant structure in most animals receiving hUCM and in all animals without SCs. Authors do not mention the percentage of implanted cells surviving and do not confirm their differentiation to myofibres or “normal” histology.

In 2010, White *et al*[[39](#_ENREF_39)] published the first randomised study with 120 Sprague-Dawley virginal female rats. The authors performed a transection of EAS with a 7-mm incision (in this specie EAS is about 3-4 mm in longitudinal length). Animals are first randomly allocated to repair or not and then each group receives injected allogeneic pre-confluence myogenic stem cells (3.2 × 106 on saline) or saline solution. If a repair is performed, a two layer 5-0 braided polyglactin interrupted suture (1 mm apart) is applied to rectal mucosa and EAS were approximated with two single interrupted stitches. Injections are applied under microscopic guidance in EAS ends (before repair if scheduled). Animals are sacrificed at 1, 3 and 13 wk and EAS contractility is studied in muscle strips *in vitro*. Contractile function was severely impaired 7 d after injury independent of repair. Twitch tension, maximal tetanic contraction, and maximal force in response to electrical stimulation improved significantly with time after sphincter repair. Injected SCs in repaired sphincters resulted in significantly superior (*P* < 0.001) contractile function at both 7 d and 90 d compared with saline. In non-repaired animals, contractile function did not improve with or without SCs. Repair and surgery could cause an deterioration in function in the short-term and indicators of denervation did not change between groups. The authors propose that SCs need some favourable conditions to work (preserved innervation, muscle apposition), as demonstrated by the minimal effect on non-repaired animals.

In the same year, Craig *et al*[[40](#_ENREF_40)] analysed the feasibility and safety of allogeneic rat myoblasts injected into the intact EAS of 4 non-pregnant female Sprague Dawley rodents. Here, 1.5 or 4.5 × 106 labelled cells, divided across three sites, were injected under electromyographic guidance between 3 and 9 o’clock. Ten days later, the authors detected labelled cells within the EAS using immunofluorescence assays. For them, this demonstrates that myoblasts integrate into the host tissue.

Also in 2010, Kajbafzadeh *et al*[[41](#_ENREF_41)] published a paper on rabbits. A surgical subtotal external sphincterotomy (9 mm longitudinal) in the posterior part through an 8-mm longitudinal incision was performed, with only the skin sutured. Three weeks later, autologous MDSCs (7 × 107, 9 animals) or saline buffer (12 animals) were injected in section borders. Sphincter electromyography (EMG) and manometry (ARM) were performed immediately before injury and 14, 28, and 60 d after injection in 3 animals per group at every interval that were sacrificed for histology studies. The three remaining animals of control group received EMG and ARM after 6 mo. Related to clinical presentation, after the injury, all rabbits demonstrated a flaccid sphincter and occasional loose faecal consistency; this persisted during the 6 mo follow-up in the control group but recovered after four weeks in the SC group. Upon histological evaluation,in the control group, circular fibres around lesion became atrophied and inflammatory infiltrate, fibrosis and a muscular gap persisted at all of the follow-ups. With SCs, myotubes appeared at 2 wk and myofibres with variable disposition at 4 wk; at 2 and 4 wk, labelled cells were detected in all of the grafted sphincters and there was less cd3+ cells infiltration at 4 wk (null at 8) and a very few by cd34+ cells appeared (so the findings cannot be explained by bone marrow derived cells infiltration). A higher proliferative index was also identified with SCs. On functional examinations, injury supposed a decrease of approximately 87% in basal pressures. ARM and EMG showed a significant (*P* > 0.001) improvement in the mean anal canal pressure and electrical activity, both at rest and after stimulation, since 4 wk after cell injection (74.8% and 60%-80% of normal values, respectively), which did not appear in the saline group. These values grew in the SC group during the evaluated period. No significant differences were noted with regard to functional and pathological parameters with time in the control group.

The following publication was issued by Pathi *et al*[[42](#_ENREF_42)] in 2012, and first compared local and systemic SCs. They performed the same injury and repair as White[[39](#_ENREF_39)] for 204 nulliparous Lewis rats compared to 20 non-injured animals. Operated rats randomly received one of the following: local and intravenous phosphate buffered saline (PBS), local allogeneic BM-MSCs (4 × 106 labelled SCs injected on each side of the reparation) with intravenous PBS and intravenous allogeneic BM-MSCs (4 × 106 labelled) with local PBS. Animals were studied at 24 and 48 h and 7 and 21 postoperative days using genetic sphincter expression by quantitative RT-PCR (IL-10, IL-6, TGFβ1, TNFα, CPH-A, COX2, LOX) and with histology and neurophysiology results at 21 d.

***Functional results (21 d)***

There was a significant decay in maximal contractile pressure and an increase in fatigue with PBS; those values were equal to those in non-operated animals in the group receiving local BM-MSCs and reached intermediate values when systemic BM-MSCs were applied. On the histological evaluation, when PBS was injected locally (independent from the systemic product), there was a muscular gap replaced by an inflammatory area with fibrosis, and skeletal muscle fibres lost their orientation in the injury borders; with local MSCs, the correct orientation appeared and fibres crossed the fibrous area. Labelled cells were detected at 24 and 48 h but not at 7 and 21 d. In wound healing parameters, pro-inflammatory (COX-2 and IL-6 during 48 h) and anti-inflammatory (IL-10 and TNFα during 21 d) increased transiently after injury in all groups, whereas TFG-β1 (an important mediator of matrix deposition by MSCs) and lysyl oxidase (related with collagen and elastin synthesis) increased significantly at earlier time points with direct MSCs and in an intermediate manner with systemic MSCs. It was of note that there was a nearly significant (*P* = 0.057) mortality increase with systemic MSCs related to pulmonary embolisms. The authors concluded that local, but not intravenous, MSCs improved contractility, matrix deposition and TGF-β1 and LOX in the acute phase.

In 2013, Salcedo *et al*[[43](#_ENREF_43)] published the first study considering pudendal nerve injury with 70 virgin female Sprague-Dawley rats. They applied Zutshi’s surgical injury model, consisting of an incision of EAS and IAS through a precise 3-4 mm incision in the perianal skin[[44](#_ENREF_44)] and pudendal nerve crush (comprising 30 seconds with a Castroviejo needle holder) as FI models. Animals were assigned randomly to: surgical sphincterotomy (*n* = 20), pudendal nerve crush (*n* = 20), sham sphincterotomy (*n* = 10, 5 seconds pressure) and sham pudendal nerve crush (*n* = 20, only dissection). Then, 24 h after injury, when they had previously demonstrated significant cytokine level changes[[45](#_ENREF_45)], they applied 2 × 106 labelledallogeneic BM-MSCs in PBS divided in each of the four sphincter quadrants or intravenously (5 animals each delivery system per experimental group) and compared them with the same volume of local or intravenous PBS. ARM and EMG were recorded immediately after injury and 10 d after treatment. The authors found that IV MSCs resulted in a significant increase in resting and peak pressure, as well as EMG amplitude and frequency at 10 d compared to PBS. Local MSCs significantly increased resting pressure and EMG frequency, but not amplitude. There were no improvements with MSCs or PBS after pudendal nerve crush, possibly due to the prompt SC administration prior to denervation changes. With sham surgery, no changes appeared in any group. Labelled cells were not found in MSC-treated animals. The authors concluded that MSCs (local or systemic) could significantly improve ARM and that IV MSCs significantly improved EMG both after sphincterotomy but not after pudendal nerve crush.

Also in 2013, Kang *et al*[[46](#_ENREF_46)] published the first experience combining bioengineering and SCs in a dog model of FI. Sphincter injury was induced by the partial extraction of 25% of the posterior IAS/EAS using electrocautery and the dogs were randomly allocated to receive or not (5 and 5, respectively) an injection of porous polycaprolactone beads containing autologous myoblasts into the injury three months later. The authors evaluated compound muscle action potentials (CMAPs) of the pudendal nerve, ARM, and histopathology 3 mo after treatment. CMAPs significantly decreased with injury (*P* = 0.04) but there were no differences between experimental groups (*P* = 0.49). Resting and squeezing pressures also significantly decreased with injury (*P* = 0.04) and were higher in the SC group, but without statistical significance. In histological analysis, in the control group, extensive damage to the muscle fibres with atrophy, cytoplasmic fibrosis and focal interstitial inflammatory cell infiltration appeared; in the therapeutic group, there was a marked foreign body reaction (numerous giant cells and foamy macrophages), with weak staining for α-smooth muscle actin. Therefore, the results did not show firm evidence that injection could improve sphincter function. In the discussion, the authors mentioned that the physical properties of some beads could elicit an adverse immune response or foreign body reaction. Also, insisted on the advantages of a large animal model to study effects (especially *in vivo*), mentioned the necessity of reinnervation and emphasised the limitation of the very low number of studied animals.

Also in 2013, Jacobs *et al*[[47](#_ENREF_47)] published the first study with a worrisome concern on safety. Here, 33 female virginal Sprague-Dawley rats received surgical anal sphincter transection and repair after which 24 underwent the injection of 5.0 × 106 allogeneic MDSCs and 9 were the sham control. SC migration to the liver and lung as well as sphincter histology were evaluated at 30 d. No evidence of SC migration to the liver or lung was found, but 2 local foci of growth were noted in two animals receiving SCs. Further evaluations of them were consistent with the benign nature: there were no nuclear abnormalities or proliferation. The authors consider that this finding could be explained by the high dose employed, cell trapping, SC overgrowth, and/or paracrine factors. Finally, they concluded that more studies are needed on safety, which could be focused locally, as no migration appeared.

Also in 2013, Bisson *et al*[[48](#_ENREF_48)] published a cryoinjury study on Fischer rats. The authors verified that the minimal lesion causing sustainable deficiency was from 90 degrees, repeated after a 24-h interval. Evaluations used an electro-stimulated ARM and histology. Experimental groups were: uninjured controls (*n* = 11), cryoinjured + PBS (*n* = 8), and cryoinjured + labelled syngeneic myoblasts injected with microscopic guidance. The novelties were the analysis of different doses and injection sites and the first long-term follow-up (6 mo): Three individual injections of 1 × 105 (*n* = 6), 1 × 106 (*n* = 8), or 1 × 107 (*n* = 6), two at the borders and the last within the lesion; alternatively, a single dose of 1 × 106 (*n* = 6) was injected into a unique site, within or opposite the lesion. Injections were well tolerated. In the histology, EAS reconstitution was observed and SCs became integrated and differentiated into mature myofibres. Related to manometry, pressures increased over time; after day 30, the SC group had significantly higher pressures compared to PBS-controls (*P* < 0.001) and equal to normal rats at day 60. The therapeutic effect persisted over a period of 6 mo. A three injection system was equally as effective as a single intra-lesion administration at day 60, but an injection opposite the lesion was unable to restore sphincter pressures.

The last publication from 2013 was from Lane *et al*[[49](#_ENREF_49)], with Sprague-Dawley rodents. They first established normative EMG EAS parameters. A more radical procedure named proctoepisiotomy with an incision length of 5mm to include transection of the IAS and EAS was designed. Then, a layered repair was performed with 6-0 delayed absorbable sutures in a running fashion followed by an interrupted layer. Animals were randomly assigned to receive myogenic SCs (*n* = 24, 5 × 106 injected under direct visualisation with a dissecting microscope one half to each side of the EAS) or PBS (control group, *n* = 9). The authors evaluated the efficacy by EMG (basal, 2 and 4 wk) and ARM (basal and 2 wk post intervention) and measurements of IAS, EAS and total sphincter thickness (millimetres) were also calculated. They found a significant difference between groups in EMG (*P* < 0.01) and ARM at 2 wk (SC group recovered basal values), but there were no differences in EMG at 4 wk (both groups returned to baseline). There were no relevant complications and measurements of sphincter muscle thickness did not differ between transplant and control rats.

The group of Elmi *et al*[[50](#_ENREF_50)] published a study in 2014 focused on SC homing and tracing, employing magnetic resonance imaging (MRI) for the first time in this field. They employed the Kazbafzadeh[[41](#_ENREF_41)] model of FI in 12 rabbits. Animals were randomly assigned to receive either ultrasmall superparamagnetic iron oxide (USPIO)–labelled 9 × 107 autologous MDSCs (experimental group) or saline (control group) at the site of damage 3 wk later. Evaluations were performed with *in vivo* MRI, EMG, and ARM, performed before, 1 h after, and 1, 2, and 4 wk after the injection. At 4 wk, sphincter sections were obtained for histology; the semi-quantitative analysis of fibrosis, desmin, iron, CD3, and CD68 was performed in two distinct regions according to either the presence (zone I) or absence (zone II) of signal loss (related to USPIO) on the MRI. Regarding MRI results, signal loss was significant at 1 h, 1 wk, and 2 wk when compared with the pre-injection signal intensity in the SC group and the maximum signal loss was detected at 1 h followed by a gradual increase during the follow-up (statistical differences at 4 wk appeared compared with those at 1 h). In the control group, there was no statistically significant difference in signal intensity at each time point. In functional evaluation, a significant improvement in pressure and electrical activity was found in the SC group after 4 wk (*P* < 0.001, 76% of basal values). In the *histological* studies, atrophic thin circular muscle fibres with fibrosis were seen in the control group whereas regenerating myofibres staining positively for desmin and clusters of iron-positive particles were detectable in the experimental group, mainly in zone I areas. A significant decrease in the fibrotic area in zone I of the therapeutic group was identified (*P* = 0.004). Minimal infiltration of CD68+ cells and mild CD3+ was reported in both groups. Therefore, iron oxide-enhanced MRI can monitor transplanted SCs.

Raghavan *et al*[[51](#_ENREF_51)] published the development and successful implantation of a bioengineered IAS employing SCs in rats in the same year. Following their studies of bioengineered IAS since 2005[[52](#_ENREF_52)], the authors created human IAS tissue constructs combining IAS circular smooth muscle cells and human enteric neuronal progenitor cells on a collagen and laminin bilayer hydrogel. Then, constructs were implanted in the perianal region of athymic rats, optimising the implantation with platelet-derived growth factor delivered through a microosmotic pump. The implantation was feasible and safe: there were no complications or rejection during the 4 wk follow-up. Implants were viable and had normal morphology, important neovascularisation, and normal *in vitro* and *in vivo* contractility and normal stooling were observed.

Another publication by the group of Salcedo and Zutshi, from the Cleveland Clinic in Ohio, one of the most important in this field, also date from 2014[[53](#_ENREF_53)]. They randomly divided 50 Sprague-Dawley rats into two groups: non-injured (*n* = 15) or injured (*n* = 35). The authors modified their prior injury model to a more aggressive one: an excision of 25% of IAS and EAS through an incision in the ventral aspect and excision from the 10 to 2 o’clock position under a dissecting microscope. They evaluated the delay to injury administration (24 h or 3 wk) of allogeneic MSCs. Non-injured animals were divided into groups that received either intrasphincteric MSCs (*n* = 8, evaluated at 10 d -5- and 5 wk -3-) or MSCs by serial *i.v.* infusions (*n* = 7, evaluated at 10 d -5- and 5 wk -2-) 24 h later. The injury group was divided into groups that received: (1) saline (*n* = 10), either locally (*n* = 5) or by serial *i.v.* infusions (*n* = 5); (2) MSCs (*n* = 10) into the sphincter (*n* = 5) or by serial *i.v.* infusions (*n* = 5); or (3) no treatment (*n* = 5), 24 h later. Rats were evaluated with ARM and immunofluorescence 10 d after treatment and at 5 wk. An additional group of 10 rats underwent local (5 rats) or *i.v.* (5 rats) application of MSCs 3 wk after injury to test the hypothesis that delayed administration will not produce SC homing because of the loss of cytokine signalling. SC administration consisted of the delivery of 5 × 105 labelled allogeneic BM-MSCs in PBS; in *i.v.* treatments, the same dosage was delivered daily for 6 consecutive days via the tail vein. Related to function, ten days after IM/IV MSC treatment, pressures were significantly increased compared with the PBS group and with pre-treatment (*P* < 0.001). At 5 wk, there were no significant differences between injury and non-injury, independent of treatment, but pressures were significantly increased after systemic or local MSC administration compared with PBS (*P* < 0.001). Related to histology, when MSCs were supplied, less of a muscular gap, and a marked decrease in fibrosis and scar tissue appeared, with the *i.v.* infusion showing the least scarring. When MSCs were administered three weeks after injury, there were only significant differences with pre-treatment values, not with the other experimental groups.

The last publication of 2014 is from Fitzwater *et al*[[54](#_ENREF_54)], and was a continuation of the White investigation using the same injury and repair procedures[[39](#_ENREF_39)] in 40 young female Sprague-Dawley rats. Animals were randomised to receive an injection of either PBS or allogeneic MDSCs at the transection site (two injections of 1.6 × 106 at each side) and then euthanised at 7 or 90 d (a half each period) for histological evaluation. The authors found sphincter disruption in 100% of both groups’ animals 7 d after injection, but 89% of controls and 78% of SCs had intact sphincters at 90 d. Striated muscle volume increased significantly from 7 to 90 d in both groups without statistical differences between them at 7 or 90 d. Significant inflammatory infiltrate was seen in both groups at 7 d, and persisted at 90 d, without any differences between groups. However, White *et al*[[39](#_ENREF_39)] observed a substantial temporal improvement in the contractility of the SC group compared with PBS, so the authors suggest that SCs might improve function without modifying histology.

In 2015, Oh *et al*[[55](#_ENREF_55)] contributed two publications about an FI model in mongrel dogs, which consisted of resecting 25% of the posterior part of both sphincters through a perianal incision; no repair was performed and treatments were administered one month after injury. In the first one[[55](#_ENREF_55)], the authors compared a control group of sham surgery (only skin incision, *n* = 5) with 10 injured dogs receiving polycaprolactone beads with PKH-26-labelled autologous myoblasts injected (*n* = 5) or PBS solution (*n* = 5). Three months later, ARM and histopathological studies were performed. Anal pressures were significantly higher in SC-treated dogs than in control dogs and the PBS group had significantly lower pressures than sham surgery dogs (*P* < 0.05). Contractile pressure in SCs dogs was 49.5% of the average before surgery, whereas it was only 32.8% in the PBS group at the same time. Immunofluorescence confirmed that some myoblasts were differentiated in all animals because labelled cells were detected and some expressed smooth and skeletal muscle markers. In their second publication[[56](#_ENREF_56)], they randomised ten injured dogs to receive either PKH-26-labeled autologous myoblasts (group A, 5 dogs) or autologous myoblasts and bFGF-loaded (basic Fibroblast Growth Factor, a muscle differentiation regulator) polycaprolactone beads (group B, 5 dogs). ARM, pudendal nerve CMAPs and histology were evaluated at three months. They found a significant improvement in ARM and CMAPs in group B compared to A (*P* = 0.002 and 0.001, respectively; in fact, both decreased in group A compared to basal values) and labelled cells were detected in 2/5 (40%) and 5/5 (100%) dogs in the A and B groups, respectively. Therefore, group B treatment improved the recovery, outcomes and SC implantation compared to cell-based therapy alone.

In the same year, Montoya *et al*[[57](#_ENREF_57)] published a bioengineering investigation with Sprague-Dawley female rats. Eighty rats underwent midline transection of both AS by a 7-mm full-thickness incision without repair. After 2 wk, the edges were re-exposed and animals were randomly assigned to receive the following treatment by injection (20 animals each one): (1) nothing (non-repaired control, NRC); (2) a polyethylene glycol-based hydrogel matrix scaffold combined with PBS (PBS/hydrogel); (3) a hydrogel matrix scaffold combined with allogeneic pre-confluence MDSCs (3.2 × 106 cells, a half in each edge, SC/hydrogel); and (4) type I collagen. Then, 4 or 12 wk later (10 and 10 animals from each group), animals were sacrificed, and their sphincters were analysed for contractile function, disruption, and striated muscle volume. Time-matched unoperated controls (UOC) were utilised for each of the two time points (*n* = 10 each period). In functional analysis, after 4 wk, maximal electrical field-stimulated contractions were significantly decreased in all four non-repaired groups compared with non-injured; however, contractions were improved in SC/hydrogel group relative to NRC (significant), PBS/hydrogel, or collagen groups. NRC and PBS/hydrogel deteriorated at 12 wk, while SC/hydrogel maintained improvement. Related to morphology, striated muscle volume increased significantly *vs* NRC from 4 to 12 wk for PBS/hydrogel (65%) and SC/hydrogel animals (63%) and at 12 wk, SC/hydrogel animals had greater striated muscle volumes than all other treatment groups (*P* = 0.001); no differences appeared at 4 wk. At 12 wk, all NRC showed disruption, while only 20% of SC/hydrogel (*P* = 0.048) and 0% of collagen-treated (*P* = 0.008) were disrupted. There was also remarkably little inflammation at 4 and 12 wk with SC/hydrogel or collagen, with occasional giant multinucleated cells and small vascular channels on intervening fibrosis between muscular endings. Therefore, a compatible matrix may facilitate SC survival, differentiation, or function, leading to functional recovery, despite morphological disruption.

In 2015, Kajbafzadeh *et al*[[58](#_ENREF_58)] published another bioengineering model with rabbits. The EAS of 16 rabbits were resected, decellularised and transplanted to the terminal rectum of the incontinent rabbits 6 mo later. Animals were divided into two groups: 1 (*n* = 8) receiving injected 7 × 107 autologous myogenic satellite cells into the implant; and 2 (*n* = 8) without injection. Histological evaluation at 3-mo intervals and EMG with electrical stimulation after 2 years (the longest follow-up published) was performed. In the histological evaluation, no evidence of inflammation or rejection was observed and the transplanted EAS appeared normal; there were no morphological differences, but all markers in the SC group immunohistochemistry revealed significant enhancement 3 and 6 mo after surgery (*P* < 0.001) without significant differences between 12 and 24 mo. In the functional evaluation, in both groups, grafted EAS contracted in response to needle and electrical signals to the muscle and the pudendal nerve; more signals were always detected in group 1, but no statistical study about this issue is provided.

In 2016, Sun *et al*[[59](#_ENREF_59)], also from Zutshi´s team, expanded the concepts of delayed repair and SCs homing further. First, the authors investigated the best electrical stimulation parameters in an SD rat model; secondly, they evaluated the most efficient delivery route for allogeneic BM-MSCs, randomly allocating SD rats into 3 groups: Intravascular (*n* = 20), intraperitoneal (*n* = 8), or direct (intramuscular) injection (*n* = 14). In both experiments, *in vivo* cytokine expression and luciferase-labelled sphincter cell imaging were employed. Only with direct intramuscular injection was a significant (*P* = 0.03) increase in MSC retention at the site of electrical stimulation found compared to sham-stimulated animals. Finally, 16 SD rats underwent a ventral excision of 50% circumference of AS and then randomly received (4 animals each group): (1) no treatment; (2) daily electrical stimulation for 3 d; (3) 3 d stimulation followed by 106 MSCs at injury site the third day; and (4) 3 d stimulation with 2 injections of 106 MSCs the first and third days three weeks later. Function was assessed before and 4 wk after intervention when histologic assessment was also done. In the results, four weeks after intervention, there was significantly more new muscle in the injured area and a significantly improved anal resting pressure in group 3 compared with all other groups.

Also in 2016, Mazzanti *et al*[[60](#_ENREF_60)] (from Lorenzi’s group) published a study with 32 Lewis rats using Lorenzi´s injury and primary repair models[[35](#_ENREF_35)]. There were four experimental groups: sphincterotomy and repair plus intrasphincteric injection of saline (A), *in vitro* expanded allogeneic BM-MSCs (B), minimally manipulated allogeneic BM mononuclear cells (MNCs, C) and the fourth underwent sham operation (D). At day 30, histologic, morphometric, *in vitro* contractility, and functional analyses were performed. Both SCs improved muscle regeneration: a large gap in the muscular layer filled with dense connective tissue and mast cells appeared in group A that is almost completely repaired in the SC groups that contained numerous small clusters of smooth muscle cells irregularly interspersed in the fibrosis. Moreover, SC groups showed increased contractile function compared to saline (*P* < 0.05). No significant difference was observed between the two SCs used. GFP+ (Green Fluorescent Protein) cells remained in the injury proximity for up to 30 d post-injection. The authors concluded that both kinds of SCs are similar in terms of efficacy.

In 2017, Sun *et al*[[61](#_ENREF_61)] published an interesting paper combining cytokines, bioengineering and SCs trying to mimic acute injury conditions homing SCs with cytokines, because healing at a time distant to injury, as in clinical situations, is a huge challenge. Thirty-two female Sprague Dawley rats underwent 50% excision of AS complex; three weeks later, 4 interventions were randomly allocated (*n* = 8): (1) no intervention; (2) 100-μg plasmid expressing stromal derived factor 1 (SDF-1); (3) plasmid and 800000 allogeneic BM-MSCs (injected at injury area); and (4) plasmid with a gelatine scaffold mixed with cells (same dose) injected 3 d later. The authors analysed ARM before and 4 wk after intervention when histology was also studied. Related to function, the 3 intervention groups had a significantly greater change in resting pressure compared with the control group. In histology, plasmid and plasmid-with-cells groups showed increased muscle mass and architectural organisation, whereas controls showed disorganised architecture and least muscle. There was also significantly less fibrosis at injury sites in the plasmid and plasmid-plus-cells groups compared with the control group. Therefore, the local delivery of SDF-1 plasmid with or without local MSCs enhanced sphincter muscle regeneration long after injury, thereby improving functional outcome.

Also in 2017, Bohl *et al*[6] developed a passive FI model in rabbits and studied bioengineered IAS. The injury consisted of an IAS hemicircumferencial sphincterectomy through a ventral curvilinear incision. Autologous biosphincter innervated constructs were produced using IAS biopsy and small bowel biopsy to obtain enteric neural SCs, employing the methodology of Gilmont *et al*[[62](#_ENREF_62)]. Six constructs were obtained from each animal and were supplemented with neural differentiation medium (Neurobasal-A). Each rabbit received four biosphincters (with 2 million smooth muscle cells and 800,000 neural progenitors). Twenty female rabbits divided in three groups were used: Non-treated (6): Injury without treatment; Treated (10): Injury followed by the implantation of biosphincters conforming a ring in the intersphincteric space 6-8 wk later (only 8 finally evaluated); and sham group (4): Injury followed by re-accessing the surgical site without more manoeuvres. ARM was used before and after injury and 1 and 3 mo after treatment; histology was also analysed. Related to function, after the injury, all rabbits had significantly decreased basal tone and loss of Recto-Anal Inhibitory Reflex (RAIR) and anal hygiene; these findings were sustained at 3 mo in groups A and C. In group B, both parameters were restored and significantly higher at 1 and 3 mo. In histological evaluation, smooth muscle reconstruction and continuity were observed in group B compared with the others; innervation and vascularisation of implants were also observed.

The same year, Sun *et al*[[63](#_ENREF_63)] hypothesised that **r**egenerating at a time remote from injury requires the re-expression of cytokines to attract SCs. Here, 56 female Sprague-Dawley animals underwent the same procedure as in their previous paper (50% ventral excision)[[59](#_ENREF_59),[61](#_ENREF_61)] and three weeks later were randomly allocated to four groups (14 animals per group): (1) no treatment; (2) 100 μg of SDF-1 plasmid injected locally; (3) local injection of plasmid and 8 × 105 BM-MSCs 3 d later; and (4) plasmid and a gelatine scaffold mixed with BM-MSCs 3 d later. Protein expression of cytokines CXCR4 and Myf5 was investigated 1 wk after treatment (*n* = 6 per group) and the resting animals received ARM, histology, immunohistochemistry and morphometry 8 wk after treatment. Related to functional results, all of the groups receiving the plasmid had significantly higher anal pressures than controls without differences between groups receiving the plasmid. Inmorphology, all of the groups receiving the plasmid had significantly more organised muscle architecture than controls without differences between therapeutic groups. Also, animals receiving plasmid alone had significantly greater muscle (smooth and skeletal) in the defect (*P* = 0.03) than either animals with injury alone (*P* = 0.02) or those receiving the plasmid, cells, and scaffold (*P* = 0.03). Significantly less fibrosis appeared with plasmid alone. There were no differences in CXCR4 or Myf5 levels at 1 wk. The authors concluded that an SDF-1 plasmid may be sufficient to repair an injured anal sphincter, even long after the injury and without MSCs or scaffold treatments.

In the first three months of 2018, three publications have appeared. The first is from our research team, and is the pioneer employing both autologous (syngenic) ASCs and biosutures for FI[[64](#_ENREF_64)]. First, anorectal normal anatomy was studied on Wistar and BDIX female rats. Then, an injury model consisting of a 1-cm extra-mucosal myotomy beginning at the anal verge in the anterior middle line was defined and characterised histologically and functionally (ARM). After injury, 36 BDIX rats were randomised to three groups for: (1) cell injection (106 labelled ASCs) without repair; (2) biosuture repair (two sutures with 1.5 × 106 GFP-ASCs); and (3) conventional suture repair and cell injection. Functional, safety and morphological studies were conducted during 1 wk. Biosutures became covered with 820000-860000 ASCs, with 100% viability, but some ASCs remained adhered after suture use. ARM showed spontaneous, consistent, rhythmic contractions, taking the form of “plateaus” with multiple twitches and being very heterogeneous in frequency, mean duration and mean number of peaks. With the injury, both sphincters were completely sectioned, and in ARM, the described activity was replaced by a gentle oscillation of basal line without a pattern. Surprisingly, these findings appeared irrespective of repair or treatment received. ASCs survived in this potentially septic area for at least 7 d: 84% of animals had GFP+ cells, mainly in the muscular section area or in interposed tissue forming "conglomerates" with injections (groups 1 and 3) or wrapping the biosutures. ASCs were also able to migrate to the damaged zone. No relevant adverse events, mortality or unexpected tissue growths were found.

The following publication was from Kuismanen *et al*[[65](#_ENREF_65)] with Sprague-Dawley rats and with the novelty of employing xenogeneic human ASCs supplemented with human platelet lysate. For injury, the authors mimicked an acute fourth grade sphincter tear sectioning both AS and anal mucosa and then repaired all plane by plane with 6-0 poliglecaprone running sutures using magnifying loupes. Injections (at 30º and 330º on a superimposed clock face) were administered prior to perianal skin closure. They also tested whether ASC efficacy could be improved by adding a polyacrylamide hydrogel carrier, Bulkamid. Female virgin rats were randomised into four groups (*n* = 14-15/group): hASCs (3 × 105) in saline or Bulkamid and saline or Bulkamid alone. Evaluation methods: ARM before and two (*n* = 58) and four weeks after injury (*n* = 33), micro-computed tomography and histology. In *functional* evaluation, both median resting and peak pressure were significantly higher at 2 and 4 wk in ASC groups compared with the other groups, and both grew more during the evaluation period; there was no difference between the ASC-carriers (saline *vs* Bulkamid). In morphological evaluation, no ASCs were recognised at either 2 or 4 wk, and there was no difference in muscle continuity, fibrosis, or collagen formation between the four groups. Bulkamid-hydrogel was well integrated with minor foreign body reaction. The inflammation was scored considering cell infiltration, oedema, haemorrhage and necrosis, as described by Nolte *et al*[[66](#_ENREF_66)], and there was significantly more inflammation in the hASC-groups, especially in the saline-ASCs. The authors also found a good correlation between histology and micro-CT, so they proposed this for non-destructive morphometric analysis on the whole injured area.

The most recent publication is from Li *et al*[[67](#_ENREF_67)], the pioneer evaluating electroacupuncture (with a galvanic stimulation) combined with SC therapy. The authors employed Zutshi’s surgical injury[[44](#_ENREF_44)] without repair. Sixty Sprague-Dawley rats were randomly divided into five groups of 12: (1) sham-operated control; (2) injured; (3) injury plus electroacupuncture (EA); (4) injury plus allogeneic BM-MSCs; and (5) injury plus BM-MSCs and EA. EA was performed once a day for 6 consecutive days by inserting an acupuncture needle bilaterally 5 mm at ST36 point and connecting them to a low-frequency electronic pulse instrument. BM-MSCs were administered with a single injection of 9.6 × 106 SCs in the caudal vein. Animals not receiving EA underwent needling at ST36 connected to an acupuncture apparatus and animals not receiving BM-MSCs were given a normal saline injection. Only morphological analyses were performed on days 1, 3, 7 and 14. In histology, BM-MSCs and EA associated with neovascularisation, fibroplasia and less inflammation and both combined obtained the strongest effects; also BM-MSCs and EA significantly increased capillary density, with the BM-MSC + EA group having the highest values. Sarcomeric α-actinin expression was significantly higher at day 14 in groups 3–5 compared to 2 (only injury), and in group 5 compared with 3 and 4 (P = 0.009 and P = 0.005 respectively), suggesting that tissue repair was higher in the BM-MSC+EA group. Similar results were observed in SDF-1 and MCP-3 expression, suggesting promotive effects of EA on the homing of BM-MSCs. The authors concluded that the combination of EA and BMSC is more effective.

In a brief analysis, there is high heterogeneity in faecal incontinence models (different surgical sections, variable partial excisions, total excision, cryoinjuries and pudendal nerve crush) and in injury managements (repair or not, substitutio). There are two mostly employed SCs: Muscle progenitors (including MDSCs and myoblasts, more committed and derived from the previous, 15 studies) and bone marrow cells (10); allogeneic or autologous use is similar (17 and 11 studies respectively, one uses both types). Muscle progenitors are less defined on literature than MSCs; there is no consensus defining MDSCs and myoblasts as opposed to MSCs and ASCs, so the cellular products employed on publications could be more heterogeneous and could combine different cell lines. Thirteen studies randomly assigned treatments. Murine models are mostly employed (mainly for accessibility and lower cost: 21 studies) but bigger animal models have grown in the last years (looking for greater human similarity: 5 studies with rabbits and 3 with dogs have been published). More than one third of published studies have explored combining SCs with bioengineering, with favourable results, and 8 have employed different adjuvants to enhance SC function, implantation or survival (2 SDF-1 and one study for each one of the following: Human platelet lysate, PDGF, bFGF, anal electrical stimulation, electroacupuncture, neural differentiation medium). The publications are summarised in Table 4.

All investigations, except two, confirm the safety and absence of relevant adverse events. There is one alert with local injection (2 local benign foci of growth in nearly 400 published injected animals)[[47](#_ENREF_47)] and another with systemic (mortality increment associated with pulmonary embolisms)[[42](#_ENREF_42)] maybe by the high doses employed.

In general, good and encouraging morphological and functional results have been observed as well as data suggesting regeneration aspects. There are only three studies[[54](#_ENREF_54),[63](#_ENREF_63),[64](#_ENREF_64)] finding no differences using SCs or control products (placebo[[54](#_ENREF_54),[64](#_ENREF_64)] or active[[63](#_ENREF_63)]) and another putting it in doubt[[61](#_ENREF_61)]. The majority have confirmed SC survival in this potentially septic area, but some have not been able to find cells holding SC labelling[[42](#_ENREF_42),[43](#_ENREF_43)]. Most publications only perform short or at least medium-term follow-up (3-6 mo), with only one long-term follow-up (2 years) published[[58](#_ENREF_58)]. There are many doubts concerning the mechanisms of action of SCs in this field.

We think that many more studies are needed to draw conclusions; to date, publications indicate safety and suggest a very interesting potential efficacy, but more are required to confirm these promising results.

**HUMAN STUDIES PUBLISHED:**

There are 7 publications regarding SC administration in humans for FI including 89 patients (55 receiving SCs). There was one study not focused on FI, one case report, three observational studies (two with the same patient cohort) and two randomised controlled trials. Employed SCs have been myoblasts (5 studies, all autologous) and ASCs (1 autologous and 1 allogeneic). An overview of these published investigations is presented in Table 5.

A Phase II study for complex perianal fistula by García-Olmo *et al*[[24](#_ENREF_24)] analysed FI in patients operated upon at their centre. Five out of 13 (38.46%) from the experimental group (fistulae treated with ASCs plus fibrin glue) had FI and 3 improved (60%) compared to 3 out of 13 (23.08%) in the control group (fibrin glue) who did not improve[[24](#_ENREF_24)]. The evaluation was purely subjective and the study was not designed to accomplish this objective so these results should be valued with caution.

The first specific publication was the observational study from Frudinger *et al*[[68](#_ENREF_68)]. The authors injected autologous myoblast in EAS from ten female patients with non-operated anterior lesions refractory to conservative treatment. Attempting to optimise SC integration, patients received anal electrical stimulation 15 min per day for 10 wk prior to implantation and 28 d after it. Cell dosage is not perfectly described; the authors performed 12-14 0.5 mL injections of a solution containing 20.16 × 106 SC/mL under ultrasonic guidance in a semi-circular array including EAS divided ends and the intervening scar. No adverse events appeared. There were significant decreases in the Wexner scale (13.7 unities), daily defecations (0.4) and incontinence episodes per week (8) at the one year follow-up. Related to *function,* voluntary pressure grew significantly at 1 and 6 mo but later decayed to basal values at 12 mo; maximal and median resting pressure also significantly decreased (7 and 6) between 6 and 12 mo. In morphology, there were no important changes in ultrasonography during the follow-up. Quality of life improved significantly during all the study. The authors concluded that the treatment is feasible, safe, well tolerated and improved symptomatology with a functional correlation. Five years later, a long-term evaluation was published[[69](#_ENREF_69)] analysing defecatory diary, blood analyses, quality of life and function annually. No adverse events or changes in blood analyses appeared. Wexner, resting and voluntary contraction pressures and overall and sub-measures of quality of life improved significantly (*P* < 0.001) for the entire evaluated period. Reduced defecation frequency and number of FI episodes also persisted for 5 years.

Romaniszyn *et al*[[70](#_ENREF_70)]published initially as a proof-of concept a case of autologous myoblasts implantation in EAS. Cells were obtained from the quadriceps and the patient had a traumatic AS rupture refractory to sphincyteroplasty and biofeedback; an 8-10 mm scar on both AS persisted and Faecal Incontinence Severity Index (FISI) scored 20 points. Here, 6 × 108 myoblasts were transplanted under ultrasonographic guidance distributed on both sides of the muscle scar, on the remaining EAS and directly into the scar. The procedure was uneventful. Controls took place every 6 wk for three visits and then after one year. FI improved from the 6th week: it disappeared to solids and soiling but persisted to flatus. Squeezing pressure also improved and in the EMG, activity started to be registered on scar area, where there was any activity before implantation. These results motivated them to perform a prospective study on 10 patients published in 2015[[71](#_ENREF_71)]. They included patients with FI of different origins with Wexner (CCI) higher than 10, low pressures but preserved reflex and innervation, and excluded Wexner 20, EAS defects higher than 90º and denervation. They implanted 3 × 108 myoblasts distributed on three injections: if a defect existed, 0.5 mL for each EAS border, 1 mL in the scar and the remaining volume in normal EAS; if there was no defect, 3 mL was distributed around the EAS ring. The follow-up was the same and was completed by 9 patients. No muscle biopsy or implantation procedure generated complications. Regarding ARM, no changes appeared at 6 wk but at 12 and 18 wk, values gradually increased (at 18 significantly). After 18 wk, significant subjective improvement was obtained in six patients (66.7%) and all patients improved in ARM, five very significantly (55.6%). On *EMG* evaluation, improvement was found in all visits with the highest values on 12 and 18 wk. Twelve months later, a deterioration of continence was reported by two of the six patients with good results at 18 wk (also present in ARM and EMG); nevertheless, mean values were still significantly better than before implantation. The remaining four (44.4%) continued with satisfactory results. The authors concluded that more studies are needed to obtain a longer response.

In 2017, a double-blind randomised clinical trial with allogeneic ASCs for sphincter defects was published by Sarveazad *et al*[[72](#_ENREF_72)]. Twenty patients were randomised but 18 were analysed (1 exclusion by cancer diagnosis before treatment and 1 lost in follow-up) in 2 groups: Both received a non-overlapping sphincteroplasty with 3-0 PDS and then received either 6 × 106 ASCs (9 patients, one half injected into each end of the muscle) or PBS (9 patients). Two months later, CCI score, endorectal sonography, and EMG were recorded. No adverse events related to SC were detected. Both groups improved Wexner scores without differences. In echography and EMG, the ratio of the area occupied by the muscle to total lesion area showed a significantly (*P* = 0.002) 7.91% increase in the SC group. *EMG* activity was significantly higher in the therapeutic group (*P* = 0.002). The authors conclude that ASCs may act as an adjuvant for surgery replacing fibrous tissue by muscle. The trial was registered at the Iranian Registry of Clinical Trials with the code IRCT2016022826316N2.

Boyer *et al*[[73](#_ENREF_73)] published a phase 2 randomised placebo-controlled study with autologous myoblasts this year. They included patients with severe FI (CCI ≥ 10), due to sphincter deficiency (single defect, multiple disruption or degeneration of EAS, lesions > 30% circumference are excluded) and refractory to medical treatment and biofeedback. In total, 24 patients were included, 12 receiving intrasphincteric injections of SCs and 12 placebo. Eight injections of 100 ± 20 × 106 SCs or placebo, into the remnant EAS and circumferentially, were performed in outpatient conditions under echography guidance. A 7-d course of antibiotics and a biofeedback re-education program of 15 d were employed and patients in the placebo group were eligible to receive frozen SCs after 1 year. The follow-up consisted of visits at 6 and 12 mo and the completion of CCI (primary endpoint) and FIQL scores, ARM, perineal electrophysiological tests, anal sonography, and MRI. Related to the primary endpoint, at 6 mo, the median CCI significantly decreased from baseline in both the therapeutic (9 *vs* 15, *P* = 0.02) and placebo (10 *vs* 15, *P* = 0.01) groups without differences between them. However, at 12 mo, the median CCI continued to ameliorate in SC group (6.5 *vs* 15, *P* = 0.006), while the effect was lost in the placebo group (14 *vs* 15, *P* = 0.35) observing a higher response rate in the SC arm (58% *vs* 8%, *P* = 0.03). After delayed rescue SC injection in the placebo group, the response rate was 60% (6/10) at 12 mo. In secondary endpoints, FIQL did not improve in the placebo arm at both 6- and 12-mo and one and two of its components significantly ameliorated in the therapeutic arm at 6 and 12 mo, respectively. No change was evidenced on sonography, MRI or electrophysiological tests at 12 mo for either arm. No relevant adverse events were identified relatable to treatment. Therefore, SCs have shown tolerance, safety, and clinical benefit at 12 mo despite a transient placebo effect at 6 mo.

In a brief analysis of these few publications, all of them confirm the implant safety, absence of relevant adverse events and the feasibility of employing SCs; of the 89 patients, 55 received SC-based therapies. Regarding results, encouraging clinical, morphological and functional results have been observed and data suggesting muscle increase have appeared. Only 10 patients from one study have surpassed a long-term evaluation[[69](#_ENREF_69)]; the habitual follow-up is one year. More randomised and comparative studies and long-term evaluations are needed to obtain efficacy conclusions.

**ONGOING CLINICAL TRIALS**

Looking at the United States National Institutes of Health worldwide clinical trials registry, accessible from [www.clinicaltrials.gov](http://www.clinicaltrials.gov), and also the EU Clinical Trials Register ([www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu)) on 1st April 2018, there were 6 registered clinical trials about stem cells therapy for FI. Surprisingly, there are no new records since a previously performed search one year before, which is not usual in SC therapies with continuous newly studies. We are going to describe them briefly:

***NCT02292628***

A Spanish phase I/II triple blinded randomised trial comparing autologous injected ASCs (40 × 106) with placebo in 16 patients.

**Inclusion:** A unique IAS defect and/or EAS (≤ 100º). CCI ≥ 12 and/or at least six episodes per month. FI for at least two years.

**Outcome measures:** The primary is serious adverse events during 12 mo and the secondary are changes in FI diary, ARM, CCI or FI Quality of Life during 12 mo.

**Actual situation:** Active but not recruiting.

***NCT02161003***

An Egyptian phase I/II non masked single group trial for children with FI after surgery for high imperforate anus.

**Treatment:** Unspecified dose of autologous MSCs injected all around the sphincter (12 points). Estimated enrolment: 50 patients.

**Outcome measures:** the *main* is FI Score at 24 wk and the secondary are clinical assessment at 12 wk; maximum daily dry interval (days 1, 30, 90); pelvic MRI and EMG at 90 and 180 d.

**Actual situation:** Unknown recruitment status, estimated completion date surpassed and not actualised since June 2014.

***NCT01011686***

Phase I trial focused on security of local autologous ASCs (the registry does not specify the dose and how) from South Korea.

**Eligible patients:** CCI ≥ 5, FI for more than 6 mo, AS continuity (ultrasound) and abnormal ARM.

**Outcome measures:** In primary there is one about efficacy (CCI) and another about safety (abnormality of laboratory and adverse events) and secondary consist of ARM and ultrasound. All these measures are evaluated at 4 wk.

**Actual situation:** Appears as “terminated” without obtaining the desired recruitment for unknown reasons (last data update in 2011). No related results have been published.

***NCT02384499***

Phase I randomised placebo-controlled, unicentric and single blinded trial with allogeneic ASCs from South Korea, two phases:Safety study: a dose escalation study: three groups of 3 patients receive 3 × 107, 6 × 107 or 9 × 107 cells/mL, respectively. Follow-up: physical examination, serologic and immunologic response test, CCI, satisfaction survey, WHO toxicity scale, adverse events, ARM and ultrasound at 1, 4, 8 wk, 4, 6, 9, and 12 mo. Response is assessed at 8 wk to select the best dosage;Efficacy test: It compares the efficacy of ASCs *vs* placebo (0.9% normal saline plus fibrin glue) with 6 patients in each group. Employs randomised, open-label and single-blind design. Clinical assessment and follow-up are identical to safety study.

**Eligible patients:** Failed medical therapy or biofeedback for more than 2 mo with CCI ≥ 8, continuous sphincter on sonography with decreased pressures on ARM. Cell therapy procedure: 6 mL of fibrin glue plus ASCs solution are prepared; 4 mL are injected at four points in IAS (3, 6, 9 and 12 h) and the other third in the EAS intermediate four positions.

**Actual situation:** The authors published the study protocol in 2017[[74](#_ENREF_74)], but the recruitment status is “unknown”, estimated study completion date has been surpassed by more than one year and the last update of the registry was on March 2015. No related results have been published.

***NCT01949922***

A non-masked Danish pilot study in 15 patients. It is not a pure SC trial because analyses injection of autologous muscle fibres and not SCs, but a small part of the fibres is used for analysing MDSCs number and thereby the regenerative potential of the sample.

**Eligible patients:** Patients with CCI ≥ 9 or affected quality of life after three months with pelvic floor muscle training.

**Outcome measures:** The primary is efficacy (CCI) and the secondary safety both at one year. Other: efficacy of pelvic floor muscle training (3 mo); improvements in quality of life, anal reflectometry, 3D ultrasound (1 year) and correlation between tissue samples regenerative potential and effects (1 year).

**Actual situation:** The recruitment status is “unknown”, completion date has passed and data not actualised within the past 2 years.

***NCT02687672***

A phase I/II trial not focused on FI from Jordan. Designed to treat chronic complete spinal cord injuries by autologous, purified CD34+ and CD133+ HSCs using bone marrow or leukapheresis as sources. The study focuses on safety and efficacy during 5-years and includes FI evaluation with a questionnaire as a secondary outcome. Currently active but non-recruiting and estimated completion is December 2021.

In a critical analysisof these “ongoing” trials, it draws attention that some of them are in a non-updated state or have been closed or cancelled without completing recruitment or for no well-defined causes. This generates some doubts about the methodology, or worse, about the efficacy or safety. Actually, no alerts have been publicised about safety, so it cannot be a real concern, but it is better to wait for new trials and the completion and publication of the ongoing trials’ results.

**DISCUSSION**

FI is a frequent, chronic and highly limiting condition affecting mainly quality of life and with very important economic implications. Its current treatment is multimodal and progressive. If conservative and pharmacological management fails, a variety of invasive procedures are available: sacral or tibial nerve stimulation, the injection of bulking agents, sphincter repairs, sphincter substitution using the gracilis muscle or an artificial device, and finally in totally refractory patients, a proximal stoma may be useful. To summarise, these procedures have moderate short-term efficacy and decreasing or unknown long-term efficacy, and many have high morbidity rates and compromised cost-effectiveness. In this context, a cellular therapy based in SCs is an attractive potential alternative.

One of the first problems to be solved in this field is how to obtain an FI model and its correlation with the clinical problem. Published literature shows a high variability of models, with the most frequently applied being surgery (23 publications), including 15 sections and 8 excisions (from 25% to 100% of the sphincter complex).

In section models, Lorenzi *et al*[[35](#_ENREF_35)] described a left lateral selective sphincterotomy without specifying the length. Zutshi’s model[[44](#_ENREF_44)] consisted of “a precise 3-4mm incision” which might not cause a total sphincter section. White *et al*[[39](#_ENREF_39)] performed a selective EAS lesion by a total section of 7 mm followed by rectal mucosa repair. We have described an anterior section of both sphincters of 1cm in length[[64](#_ENREF_64)], one of the most extensive sections. Going further, Lane *et al*[[49](#_ENREF_49)] performed a more aggressive injury defined as a “proctoepisiotomy” but did not describe the technical details and extension. Similar or minor modified procedures have been employed by Mazzanti[[60](#_ENREF_60)], Salcedo[[75](#_ENREF_75)], Elmi[[50](#_ENREF_50)], Pathi[[42](#_ENREF_42)], Fitzwater[[54](#_ENREF_54)], Montoya[[57](#_ENREF_57)], Kuismanen[[65](#_ENREF_65)] and Li[[67](#_ENREF_67)].

A Salcedo publication of 2010[[75](#_ENREF_75)], found that rats receiving Zutshi’s injury without repair or treatment presented anal pressure recovery 14 d later, comparable registries to controls after 28 d and bridging fibrosis on histology; these findings were not observed with pudendal nerve transection. This publication made Zutshi’s team from Cleveland Clinic Ohio turn to more aggressive procedures: Salcedo *et al*[[53](#_ENREF_53)]described an excision of 25% of both sphincters (through an incision in the ventral aspect, from the 10 to 2 o’clock position) and later Sun extended it to an excision of 50%[[59](#_ENREF_59)]. A similar model of partial excision was employed in dogs by Kang[[46](#_ENREF_46)] and Oh[[55](#_ENREF_55),[56](#_ENREF_56)] and in rabbits by Bohl[[76](#_ENREF_76)], and a total excision was employed by Kajbafzadeh[[58](#_ENREF_58)]. Aiming to minimise the effects of the poorly developed sphincters in rats, some authors have employed bigger animals such as rabbits[[38](#_ENREF_38),[41](#_ENREF_41),[50](#_ENREF_50),[58](#_ENREF_58),[76](#_ENREF_76)] and dogs[[46](#_ENREF_46),[55](#_ENREF_55),[56](#_ENREF_56)], but there are only eight published papers compared to twenty-one in rats. Other FI model are cryoinjuries employed by Kang *et al*[[36](#_ENREF_36)] (they did not specify the muscular volume damaged) and by Bisson *et al*[[48](#_ENREF_48)] who published that the minimal significant cryoinjury must be of at least 90º.

We can discuss the clinical relevance of these models. Human obstetric trauma is more complex than a simple section during episiotomy or a perineum tear. Sphincter injury may be related to muscle injury, prolonged regional hypoxia, denervation, faulty repair, or a combination of them. Other factors could be added later in life: ageing, hormonal changes, surgeries, etc. In an effort to reproduce these complex effects, simulated childbirth injury models have been designed. It was first described for urinary incontinence by Resplande *et al*[[77](#_ENREF_77)] and by Sievert *et al*[[78](#_ENREF_78)] inserting a inflated 10F Foley catheter inside the vagina for 3-4 h; to simulate labour, an episiotomy and balloon extraction was performed. Later, Healy *et al*[[79](#_ENREF_79)] published a model for FI using two intrapelvic, retrouterine balloons (6 Fr urinary catheters) placed through a 3cm laparotomy for one hour. Maybe more studies on simulated childbirth models are needed.

On the other hand, in clinical settings it is known that immediate repair offers better results, but the most frequent scenario is a repair indicated years later and with chronic local conditions (fibrosis, denervation, atrophies, no inflammation). Thus, in preclinical papers, there are 9 delayed treatments (repairing or injections delayed two[[38](#_ENREF_38)], three[[41](#_ENREF_41),[46](#_ENREF_46),[50](#_ENREF_50),[53](#_ENREF_53)] or four weeks[[55](#_ENREF_55),[56](#_ENREF_56)] and substitutions delayed 6-8 wk[[76](#_ENREF_76)] or 6 mo[[58](#_ENREF_58)]). We may discuss whether the considered periods are sufficient to mimic the chronic setting, as it is likely that only acute and subacute conditions have been tested. In the acute setting, some potential confounding factors have been observed[[64](#_ENREF_64)] (mucosal tears, faecal contamination, etc.) that could compromise SCs survivorship or effects. However, there are also cytokines fundamental for SC homing and activation as it has been demonstrated for acute myocardial infarction with the SDF-1 factor[[80](#_ENREF_80)]. In this field, Salcedo *et al*[[45](#_ENREF_45)] made some relevant contributions; they studied Stromal derived factor-1 (SDF-1 or CXCL12) and monocyte chemotactic protein-3 (MCP-3), known signals forcing homing of BM-MSCs to ischaemic tissue, expression following direct injury to the AS and pudendal nerve. They found that direct injury resulted in higher levels soon after injury and for 3 wk, whereas denervation resulted in an overexpression for only 10 d which may lead to more fibrosis[[45](#_ENREF_45)]. So, in chronic conditions, these molecules will be in normal values and this could make it difficult for SCs to act. To increase these factors, SCs could be transfected with plasmids or the local production could be stimulated using surgical injury or electrical stimulation such as in the paper by Sun[[59](#_ENREF_59)]. The previously mentioned publications[[61](#_ENREF_61),[63](#_ENREF_63)] open up an interesting new research field: the combination of SCs or their vehicles (for example sutures with Vascular Endothelial Growing Factor[[81](#_ENREF_81)]) with cytokines and growth factors.

Another problem to be solved is how to obtain better SC delivery, survival and function in tissues. All studies except ours have employed cell injections; we thought that biosutures[[31-33](#_ENREF_31)] could be useful for depositing SCs at the focus of the injury. Other authors have made different modifications to biosutures: Yao *et al*[[82](#_ENREF_82)] added poly-L-lysine and fibronectin to improve cell adherence and Horváthy *et al*[[83](#_ENREF_83)] observed better BM-MSC adherence if suture was previously covered with albumin and SC survivor in implanted tissues at 5 wk. No evidence exists about the best dose or at least the minimal “clinically active” dose. With 1.5 × 106 ASCs it was found that SCs tend to form “clusters” over the suture, in culture medium and remained adhered after biosuture usage[[64](#_ENREF_64)]. Therefore, more studies on suture preparation are needed. Delivered doses could be more controlled by injection, but a similar phenomenon can sometimes be observed: Similar clusters appear outside muscle layer with the consequent cellular loss[[64](#_ENREF_64)]. Injected doses have been very variable on published literature. To improve survival and function in tissues, the employed strategies have been the use of growth factors and cytokines, as mentioned before; this field would be very interesting in the future.

Related to the mechanism of action of SCs, there are many remaining questions. The first to be solved is whether SCs survive, integrate and participate in regeneration. More studies to identify critical pathways that are dysregulated in tissue repair are needed. Studies with myogenic cells have detected the labelling on muscle in acute and subacute phase, but medium- or long-term incorporation or the differentiation of BM derived cells has not been clearly identified, and regeneration is at least doubtful. It is possible that myogenic SCs have a greater role based on differentiation, but MSCs probably base their role much more on immunomodulation, and anti-inflammatory and angiogeneic capabilities. There is growing evidence of the immunomodulation capability of MSCs, which is thought to be largely based on inhibition of the T-cell and B-cell proliferation and dendritic cell maturation[[84](#_ENREF_84)] and in the secretion of a large number of cytokines and growth factors[[85](#_ENREF_85)]. Nemeth *et al*[[86](#_ENREF_86)] observed MSC sepsis attenuation by macrophage reprogramming to increase IL-10, a cytokine that decreases neutrophil migration. Our research team has added contributions to that evidence: Georgiev-Hristov found an early swift from acute to chronic inflammation in the presence of ASCs (neutrophil descent and macrophage increment) after tracheal anastomosis[[33](#_ENREF_33)], and Riera observed less acute and chronic inflammation during 3 mo, and the increasing fibrosis of the aneurysm sac in pigs[[87](#_ENREF_87)]. Regeneration is not clearly demonstrated in many studies and is very difficult to observe; it may be that more complex morphometric or molecular analyses are needed to confirm it. Similar studies would be applied also for another mechanism as immunomodulation (studying the amount of different cells and molecules). In fact, there are some remaining barriers to achieve “regeneration” with SCs: We need to teach them how to differentiate in an efficient manner, then, possibly with tissue engineering, we need to integrate them in an appropriate delivery system, and finally generate a blood supply and innervation that is sufficient to allow their engraftment and survival.

The last critical question is about safety; although there are other potential side effects, the most worrisome is possible carcinogenicity. SCs have surpassed preclinical studies on biodistribution and toxicity but investigations into tumour formation are still ongoing. Some publications have observed that MSCs cultured for a long time may develop malignant changes and even tumours in mice[[88](#_ENREF_88)]. However, subsequent publications, including from the same authors, attributed those findings to tumour cell cross-contamination[[89](#_ENREF_89),90], other studies did not detect it under extreme culture conditions and it has never been observed *in vivo*. In fact, the relationship between SCs and tumours is contradictory. No direct MSC transformation has been observed but there is a consensus that MSCs have enhanced tropism toward tumours and have pro-tumour (growing, angiogenesis, participation on microenvironment, immunomodulation)[[91](#_ENREF_91),[92](#_ENREF_92)] and anti-tumour (apoptosis, proliferation inhibition)[[93](#_ENREF_93),[94](#_ENREF_94)] properties. This relationship depends on lots of factors like, type of MSCs, its source, type of cancer cell line, *in vivo* or *in vitro* conditions, factors secreted by MSCs and interactions between MSCs, host immune cells and cancer cells. A possible key factor on these effects is time relation: When MSCs are administered with an existing tumour, a suppressive effect has been observed[[95](#_ENREF_95)], but in some studies with co-administration of SCs and tumour cells, tumour growth was higher compared to tumour cells alone[[96](#_ENREF_96)]. These complex interactions have been studied by several authors and reviewed by Ramdasi *et al*[[97](#_ENREF_97)]. Tropism to tumours has been exploited to treat tumours in experimental models, as reviewed by Chulpanova *et al*[[98](#_ENREF_98)]. Moreover, a recent NEJM paper published the first severe adverse event potentially relatable to ASCs: Three women suffering from macular degeneration, after undergoing ASC therapies developed complications including vision loss, detached retinas and bleeding and are now totally blind (although the ASCs were mixed with blood plasma and large numbers of platelets)[[99](#_ENREF_99)]. In conclusion, cumulated experience seems to warrant oncogenic safety of SCs, but more studies and long-term follow-up are needed to exclude definitively all the risks.

An in depth analysis about published literature has been provided at the end of each chapter. The 29 published animal investigations confirm the safety (except one) and generally good morphological and functional results appeared with remaining questions about SCs survival, effect, long-term results, efficacy on chronic conditions… In human research, there is one unrelated study[[24](#_ENREF_24)], six studies involving 55 patients receiving SCs with promising results[[68-73](#_ENREF_68)] and 6 ongoing clinical trials. More highly rigorous investigations (related to SC type, dosage, deliver system, adjuvant factors, safet) are needed previously that SC therapy for FI became a clinical reality, if efficacy and safety are confirmed.

To finish, regenerative strategies use costly culture-expansion procedures that require Good Manufacture Practice laboratories compromising cost-effectiveness, as has been demonstrated in a recent survey of clinicians about SC therapies adoption[[100](#_ENREF_100)]. It is very difficult to estimate the real potential cost of this kind of therapy for humans because there is no consensus in the type of SC, autologous or allogeneic use, the required dose, *etc*. The real efficacy needs yet to be clarified, but if a cure could be achieved, direct and indirect costs mentioned before, could disappear and probably hospitalization costs might be lower due to less invasive procedures to implant SCs compared with FI surgery. Based on our previous experience in clinical trials for anal fistulae[[22-29](#_ENREF_22)], approximated costs in Spain are the following: 1500-2500€ (1727.8 to 2879.73 USD) for closed system SVF, 2800-4000€ (3225.48-4607.83$) for 40x106 autologous ASCs and 3500-5000€ (4032.88-5761.26$) for 100 × 106 allogeneic ASCs; the costs for other MSCs are equivalent. It must be taken into account that these costs are for SCs produced and dedicated to research, and not for commercial use (maybe higher at least during the firsts years). Between 2018 and 2019 is expected the first allogeneic ASCs medicine product for fistula marketing so we will be able to know real costs on a large scale production. Moreover, some publications have reported acceptable results with free autologous muscle grafts in FI in children[[101](#_ENREF_101)] (grafts also contains SCs such as satellite cells, but the processing is easier and cheaper), opening up a new field for study.

# CONCLUSION

FI is frequent and the available treatments have improvable outcomes so alternative treatments are needed. Regenerative therapies have exciting potential to improve patient outcomes and different strategies have been explored (with or without biomaterials) in preclinical and clinical studies.

In preclinical studies, SCs derived from muscle, bone marrow and adipose tissue have been most intensively investigated. In general, safety seems to be guaranteed and some encouraging results have been observed. Clinical evidence is very limited, but the therapy appears to be safe and may be effective. More data are necessary; to date, no SC-based therapy is yet ready for ordinary clinical use as both short-term and long-term efficacy and safety have to be firmly established. More knowledge about SC and healing biology and engineering principles is needed before regenerative medicine for FI could become fact.

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**REFERENCES**

1 **Macmillan AK**, Merrie AE, Marshall RJ, Parry BR. The prevalence of fecal incontinence in community-dwelling adults: a systematic review of the literature. *Dis Colon Rectum* 2004; **47**: 1341-1349 [PMID: 15484348 DOI: 10.1007/s10350-004-0593-0]

2 **Nelson R**, Norton N, Cautley E, Furner S. Community-based prevalence of anal incontinence. *JAMA* 1995; **274**: 559-561 [PMID: 7629985 DOI: 10.1001/jama.1995.03530070057030]

3 **Edwards NI**, Jones D. The prevalence of faecal incontinence in older people living at home. *Age Ageing* 2001; **30**: 503-507 [PMID: 11742780 DOI: 10.1093/ageing/30.6.503]

4 **Dunivan GC**, Heymen S, Palsson OS, von Korff M, Turner MJ, Melville JL, Whitehead WE. Fecal incontinence in primary care: prevalence, diagnosis, and health care utilization. *Am J Obstet Gynecol* 2010; **202**: 493.e1-493.e6 [PMID: 20223447 DOI: 10.1016/j.ajog.2010.01.018]

5 **Varma MG**, Brown JS, Creasman JM, Thom DH, Van Den Eeden SK, Beattie MS, Subak LL; Reproductive Risks for Incontinence Study at Kaiser (RRISK) Research Group. Fecal incontinence in females older than aged 40 years: who is at risk? *Dis Colon Rectum* 2006; **49**: 841-851 [PMID: 16741640 DOI: 10.1007/s10350-006-0535-0]

6 **Parés D**, Vial M, Bohle B, Maestre Y, Pera M, Roura M, Comas M, Sala M, Grande L. Prevalence of faecal incontinence and analysis of its impact on quality of life and mental health. *Colorectal Dis* 2011; **13**: 899-905 [PMID: 20394640 DOI: 10.1111/j.1463-1318.2010.02281.x]

7 **Deutekom M**, Dobben AC, Dijkgraaf MG, Terra MP, Stoker J, Bossuyt PM. Costs of outpatients with fecal incontinence. *Scand J Gastroenterol* 2005; **40**: 552-558 [PMID: 16036507 DOI: 10.1080/00365520510012172]

8 **Dudding TC**, Vaizey CJ, Kamm MA. Obstetric anal sphincter injury: incidence, risk factors, and management. *Ann Surg* 2008; **247**: 224-237 [PMID: 18216527 DOI: 10.1097/SLA.0b013e318142cdf4]

9 **Oberwalder M**, Connor J, Wexner SD. Meta-analysis to determine the incidence of obstetric anal sphincter damage. *Br J Surg* 2003; **90**: 1333-1337 [PMID: 14598410 DOI: 10.1002/bjs.4369]

10 **Madoff RD**. Surgical treatment options for fecal incontinence. *Gastroenterology* 2004; **126**: S48-S54 [PMID: 14978638 DOI: 10.1053/j.gastro.2003.10.015]

11 **Halverson AL**, Hull TL. Long-term outcome of overlapping anal sphincter repair. *Dis Colon Rectum* 2002; **45**: 345-348 [PMID: 12068192 DOI: 10.1007/s10350-004-6180-6]

12 **Glasgow SC**, Lowry AC. Long-term outcomes of anal sphincter repair for fecal incontinence: a systematic review. *Dis Colon Rectum* 2012; **55**: 482-490 [PMID: 22426274 DOI: 10.1097/DCR.0b013e3182468c22]

13 **Chivu-Economescu M**, Rubach M. Hematopoietic Stem Cells Therapies. *Curr Stem Cell Res Ther* 2017; **12**: 124-133 [PMID: 26496888 DOI: 10.2174/1574888X10666151026114241]

14 **García-Gómez I**, Elvira G, Zapata AG, Lamana ML, Ramírez M, Castro JG, Arranz MG, Vicente A, Bueren J, García-Olmo D. Mesenchymal stem cells: biological properties and clinical applications. *Expert Opin Biol Ther* 2010; **10**: 1453-1468 [PMID: 20831449 DOI: 10.1517/14712598.2010.519333]

15 **Tsuchiya A**, Kojima Y, Ikarashi S, Seino S, Watanabe Y, Kawata Y, Terai S. Clinical trials using mesenchymal stem cells in liver diseases and inflammatory bowel diseases. *Inflamm Regen* 2017; **37**: 16 [PMID: 29259715 DOI: 10.1186/s41232-017-0045-6]

16 **Majka M**, Sułkowski M, Badyra B, Musiałek P. Concise Review: Mesenchymal Stem Cells in Cardiovascular Regeneration: Emerging Research Directions and Clinical Applications. *Stem Cells Transl Med* 2017; **6**: 1859-1867 [PMID: 28836732 DOI: 10.1002/sctm.16-0484]

17 **Mizuno H**, Tobita M, Uysal AC. Concise review: Adipose-derived stem cells as a novel tool for future regenerative medicine. *Stem Cells* 2012; **30**: 804-810 [PMID: 22415904 DOI: 10.1002/stem.1076]

18 **Trebol Lopez J**, Georgiev Hristov T, García-Arranz M, García-Olmo D. Stem cell therapy for digestive tract diseases: current state and future perspectives. *Stem Cells Dev* 2011; **20**: 1113-1129 [PMID: 21187000 DOI: 10.1089/scd.2010.0277]

19 **Ma T**, Sun J, Zhao Z, Lei W, Chen Y, Wang X, Yang J, Shen Z. A brief review: adipose-derived stem cells and their therapeutic potential in cardiovascular diseases. *Stem Cell Res Ther* 2017; **8**: 124 [PMID: 28583198 DOI: 10.1186/s13287-017-0585-3]

20 **González MA**, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 2009; **136**: 978-989 [PMID: 19135996 DOI: 10.1053/j.gastro.2008.11.041]

21 **Gonzalez-Rey E**, Anderson P, González MA, Rico L, Büscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. *Gut* 2009; **58**: 929-939 [PMID: 19136511 DOI: 10.1136/gut.2008.168534]

22 **García-Olmo D**, García-Arranz M, García LG, Cuellar ES, Blanco IF, Prianes LA, Montes JA, Pinto FL, Marcos DH, García-Sancho L. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis* 2003; **18**: 451-454 [PMID: 12756590 DOI: 10.1007/s00384-003-0490-3]

23 **García-Olmo D**, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005; **48**: 1416-1423 [PMID: 15933795 DOI: 10.1007/s10350-005-0052-6]

24 **Garcia-Olmo D**, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 2009; **52**: 79-86 [PMID: 19273960 DOI: 10.1007/DCR.0b013e3181973487]

25 **Herreros MD**, Garcia-Arranz M, Guadalajara H, De-La-Quintana P, Garcia-Olmo D; FATT Collaborative Group. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula Advanced Therapy Trial 1) and long-term evaluation. *Dis Colon Rectum* 2012; **55**: 762-772 [PMID: 22706128 DOI: 10.1097/DCR.0b013e318255364a]

26 **Guadalajara H**, Herreros D, De-La-Quintana P, Trebol J, Garcia-Arranz M, Garcia-Olmo D. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis* 2012; **27**: 595-600 [PMID: 22065114 DOI: 10.1007/s00384-011-1350-1]

27 **de la Portilla F**, Alba F, García-Olmo D, Herrerías JM, González FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 2013; **28**: 313-323 [PMID: 23053677 DOI: 10.1007/s00384-012-1581-9]

28 **Panés J**, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, Grimaud JC, de la Portilla F, Goldin E, Richard MP, Leselbaum A, Danese S; ADMIRE CD Study Group Collaborators. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet* 2016; **388**: 1281-1290 [PMID: 27477896 DOI: 10.1016/S0140-6736(16)31203-X]

29 **García-Arranz M**, Herreros MD, González-Gómez C, de la Quintana P, Guadalajara H, Georgiev-Hristov T, Trébol J, Garcia-Olmo D. Treatment of Crohn's-Related Rectovaginal Fistula With Allogeneic Expanded-Adipose Derived Stem Cells: A Phase I-IIa Clinical Trial. *Stem Cells Transl Med* 2016; **5**: 1441-1446 [PMID: 27412883 DOI: 10.5966/sctm.2015-0356]

30 **Oyama Y**, Craig RM, Traynor AE, Quigley K, Statkute L, Halverson A, Brush M, Verda L, Kowalska B, Krosnjar N, Kletzel M, Whitington PF, Burt RK. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. *Gastroenterology* 2005; **128**: 552-563 [PMID: 15765390]

31 **Pascual I**, de Miguel GF, Gómez-Pinedo UA, de Miguel F, Arranz MG, García-Olmo D. Adipose-derived mesenchymal stem cells in biosutures do not improve healing of experimental colonic anastomoses. *Br J Surg* 2008; **95**: 1180-1184 [PMID: 18690635 DOI: 10.1002/bjs.6242]

32 **Pascual I**, Fernández de Miguel G, García Arranz M, García-Olmo D. Biosutures improve healing of experimental weak colonic anastomoses. *Int J Colorectal Dis* 2010; **25**: 1447-1451 [PMID: 20544210 DOI: 10.1007/s00384-010-0952-3]

33 **Georgiev-Hristov T**, García-Arranz M, García-Gómez I, García-Cabezas MA, Trébol J, Vega-Clemente L, Díaz-Agero P, García-Olmo D. Sutures enriched with adipose-derived stem cells decrease the local acute inflammation after tracheal anastomosis in a murine model. *Eur J Cardiothorac Surg* 2012; **42**: e40-e47 [PMID: 22689184 DOI: 10.1093/ejcts/ezs357]

34 **Gräs S**, Tolstrup CK, Lose G. Regenerative medicine provides alternative strategies for the treatment of anal incontinence. *Int Urogynecol J* 2017; **28**: 341-350 [PMID: 27311602 DOI: 10.1007/s00192-016-3064-y]

35 **Lorenzi B**, Pessina F, Lorenzoni P, Urbani S, Vernillo R, Sgaragli G, Gerli R, Mazzanti B, Bosi A, Saccardi R, Lorenzi M. Treatment of experimental injury of anal sphincters with primary surgical repair and injection of bone marrow-derived mesenchymal stem cells. *Dis Colon Rectum* 2008; **51**: 411-420 [PMID: 18224375 DOI: 10.1007/s10350-007-9153-8]

36 **Kang SB**, Lee HN, Lee JY, Park JS, Lee HS, Lee JY. Sphincter contractility after muscle-derived stem cells autograft into the cryoinjured anal sphincters of rats. *Dis Colon Rectum* 2008; **51**: 1367-1373 [PMID: 18536965 DOI: 10.1007/s10350-008-9360-y]

37 **Saihara R**, Komuro H, Urita Y, Hagiwara K, Kaneko M. Myoblast transplantation to defecation muscles in a rat model: a possible treatment strategy for fecal incontinence after the repair of imperforate anus. *Pediatr Surg Int* 2009; **25**: 981-986 [PMID: 19690871 DOI: 10.1007/s00383-009-2454-3]

38 **Aghaee-Afshar M**, Rezazadehkermani M, Asadi A, Malekpour-Afshar R, Shahesmaeili A, Nematollahi-mahani SN. Potential of human umbilical cord matrix and rabbit bone marrow-derived mesenchymal stem cells in repair of surgically incised rabbit external anal sphincter. *Dis Colon Rectum* 2009; **52**: 1753-1761 [PMID: 19966609 DOI: 10.1007/DCR.0b013e3181b55112]

39 **White AB**, Keller PW, Acevedo JF, Word RA, Wai CY. Effect of myogenic stem cells on contractile properties of the repaired and unrepaired transected external anal sphincter in an animal model. *Obstet Gynecol* 2010; **115**: 815-823 [PMID: 20308844 DOI: 10.1097/AOG.0b013e3181d56cc5]

40 **Craig JB**, Lane FL, Nistor G, Motakef S, Pham QA, Keirstead H. Allogenic myoblast transplantation in the rat anal sphincter. *Female Pelvic Med Reconstr Surg* 2010; **16**: 205-208 [PMID: 22453342 DOI: 10.1097/SPV.0b013e3181ec1edd]

41 **Kajbafzadeh AM**, Elmi A, Talab SS, Esfahani SA, Tourchi A. Functional external anal sphincter reconstruction for treatment of anal incontinence using muscle progenitor cell auto grafting. *Dis Colon Rectum* 2010; **53**: 1415-1421 [PMID: 20847624 DOI: 10.1007/DCR.0b013e3181e53088]

42 **Pathi SD**, Acevedo JF, Keller PW, Kishore AH, Miller RT, Wai CY, Word RA. Recovery of the injured external anal sphincter after injection of local or intravenous mesenchymal stem cells. *Obstet Gynecol* 2012; **119**: 134-144 [PMID: 22183221 DOI: 10.1097/AOG.0b013e3182397009]

43 **Salcedo L**, Mayorga M, Damaser M, Balog B, Butler R, Penn M, Zutshi M. Mesenchymal stem cells can improve anal pressures after anal sphincter injury. *Stem Cell Res* 2013; **10**: 95-102 [PMID: 23147650 DOI: 10.1016/j.scr.2012.10.002]

44 **Zutshi M**, Salcedo LB, Zaszczurynski PJ, Hull TL, Butler RS, Damaser MS. Effects of sphincterotomy and pudendal nerve transection on the anal sphincter in a rat model. *Dis Colon Rectum* 2009; **52**: 1321-1329 [PMID: 19571711 DOI: 10.1007/DCR.0b013e31819f746d]

45 **Salcedo L**, Sopko N, Jiang HH, Damaser M, Penn M, Zutshi M. Chemokine upregulation in response to anal sphincter and pudendal nerve injury: potential signals for stem cell homing. *Int J Colorectal Dis* 2011; **26**: 1577-1581 [PMID: 21706136 DOI: 10.1007/s00384-011-1269-6]

46 **Kang SB**, Lee HS, Lim JY, Oh SH, Kim SJ, Hong SM, Jang JH, Cho JE, Lee SM, Lee JH. Injection of porous polycaprolactone beads containing autologous myoblasts in a dog model of fecal incontinence. *J Korean Surg Soc* 2013; **84**: 216-224 [PMID: 23577316 DOI: 10.4174/jkss.2013.84.4.216]

47 **Jacobs SA**, Lane FL, Pham QA, Nistor G, Robles R, Chua C, Boubion B, Osann K, Keirstead H. Safety assessment of myogenic stem cell transplantation and resulting tumor formation. *Female Pelvic Med Reconstr Surg* 2013; **19**: 362-368 [PMID: 24165451 DOI: 10.1097/SPV.0000000000000035]

48 **Bisson A**, Fréret M, Drouot L, Jean L, Le Corre S, Gourcerol G, Doucet C, Michot F, Boyer O, Lamacz M. Restoration of anal sphincter function after myoblast cell therapy in incontinent rats. *Cell Transplant* 2015; **24**: 277-286 [PMID: 24143883 DOI: 10.3727/096368913X674053]

49 **Lane FL**, Jacobs SA, Craig JB, Nistor G, Markle D, Noblett KL, Osann K, Keirstead H. In vivo recovery of the injured anal sphincter after repair and injection of myogenic stem cells: an experimental model. *Dis Colon Rectum* 2013; **56**: 1290-1297 [PMID: 24105005 DOI: 10.1097/DCR.0b013e3182a4adfb]

50 **Elmi A**, Kajbafzadeh AM, Oghabian MA, Talab SS, Tourchi A, Khoei S, Rafie B, Esfahani SA. Anal sphincter repair with muscle progenitor cell transplantation: serial assessment with iron oxide-enhanced MRI. *AJR Am J Roentgenol* 2014; **202**: 619-625 [PMID: 24555600 DOI: 10.2214/AJR.13.11146]

51 **Raghavan S**, Miyasaka EA, Gilmont RR, Somara S, Teitelbaum DH, Bitar KN. Perianal implantation of bioengineered human internal anal sphincter constructs intrinsically innervated with human neural progenitor cells. *Surgery* 2014; **155**: 668-674 [PMID: 24582493 DOI: 10.1016/j.surg.2013.12.023]

52 **Hecker L**, Baar K, Dennis RG, Bitar KN. Development of a three-dimensional physiological model of the internal anal sphincter bioengineered in vitro from isolated smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G188-G196 [PMID: 15774939 DOI: 10.1152/ajpgi.00335.2004]

53 **Salcedo L**, Penn M, Damaser M, Balog B, Zutshi M. Functional outcome after anal sphincter injury and treatment with mesenchymal stem cells. *Stem Cells Transl Med* 2014; **3**: 760-767 [PMID: 24797828 DOI: 10.5966/sctm.2013-0157]

54 **Fitzwater JL**, Grande KB, Sailors JL, Acevedo JF, Word RA, Wai CY. Effect of myogenic stem cells on the integrity and histomorphology of repaired transected external anal sphincter. *Int Urogynecol J* 2015; **26**: 251-256 [PMID: 25253391 DOI: 10.1007/s00192-014-2496-5]

55 **Oh HK**, Lee HS, Lee JH, Oh SH, Lim JY, Ahn S, Hwang JY, Kang SB. Functional and histological evidence for the targeted therapy using biocompatible polycaprolactone beads and autologous myoblasts in a dog model of fecal incontinence. *Dis Colon Rectum* 2015; **58**: 517-525 [PMID: 25850839 DOI: 10.1097/DCR.0000000000000346]

56 **Oh HK**, Lee HS, Lee JH, Oh SH, Lim JY, Ahn S, Kang SB. Coadministration of basic fibroblast growth factor-loaded polycaprolactone beads and autologous myoblasts in a dog model of fecal incontinence. *Int J Colorectal Dis* 2015; **30**: 549-557 [PMID: 25592048 DOI: 10.1007/s00384-015-2121-1]

57 **Montoya TI**, Acevedo JF, Smith B, Keller PW, Sailors JL, Tang L, Word RA, Wai CY. Myogenic stem cell-laden hydrogel scaffold in wound healing of the disrupted external anal sphincter. *Int Urogynecol J* 2015; **26**: 893-904 [PMID: 25644049 DOI: 10.1007/s00192-014-2620-6]

58 **Kajbafzadeh AM**, Kajbafzadeh M, Sabetkish S, Sabetkish N, Tavangar SM. Tissue-Engineered External Anal Sphincter Using Autologous Myogenic Satellite Cells and Extracellular Matrix: Functional and Histological Studies. *Ann Biomed Eng* 2016; **44**: 1773-1784 [PMID: 26424474 DOI: 10.1007/s10439-015-1468-3]

59 **Sun L**, Yeh J, Xie Z, Kuang M, Damaser MS, Zutshi M. Electrical Stimulation Followed by Mesenchymal Stem Cells Improves Anal Sphincter Anatomy and Function in a Rat Model at a Time Remote From Injury. *Dis Colon Rectum* 2016; **59**: 434-442 [PMID: 27050606 DOI: 10.1097/DCR.0000000000000548]

60 **Mazzanti B**, Lorenzi B, Borghini A, Boieri M, Ballerini L, Saccardi R, Weber E, Pessina F. Local injection of bone marrow progenitor cells for the treatment of anal sphincter injury: in-vitro expanded versus minimally-manipulated cells. *Stem Cell Res Ther* 2016; **7**: 85 [PMID: 27328811 DOI: 10.1186/s13287-016-0344-x]

61 **Sun L**, Xie Z, Kuang M, Penn M, Damaser MS, Zutshi M. Regenerating the Anal Sphincter: Cytokines, Stem Cells, or Both? *Dis Colon Rectum* 2017; **60**: 416-425 [PMID: 28267010 DOI: 10.1097/DCR.0000000000000783]

62 **Gilmont RR**, Raghavan S, Somara S, Bitar KN. Bioengineering of physiologically functional intrinsically innervated human internal anal sphincter constructs. *Tissue Eng Part A* 2014; **20**: 1603-1611 [PMID: 24328537 DOI: 10.1089/ten.TEA.2013.0422]

63 **Sun L**, Kuang M, Penn M, Damaser MS, Zutshi M. Stromal Cell-Derived Factor 1 Plasmid Regenerates Both Smooth and Skeletal Muscle After Anal Sphincter Injury in the Long Term. *Dis Colon Rectum* 2017; **60**: 1320-1328 [PMID: 29112569 DOI: 10.1097/DCR.0000000000000940]

64 **Trébol J**, Georgiev-Hristov T, Vega-Clemente L, García-Gómez I, Carabias-Orgaz A, García-Arranz M, García-Olmo D. Rat model of anal sphincter injury and two approaches for stem cell administration. *World J Stem Cells* 2018; **10**: 1-14 [PMID: 29391927 DOI: 10.4252/wjsc.v10.i1.1]

65 **Kuismanen K**, Juntunen M, Narra Girish N, Tuominen H, Huhtala H, Nieminen K, Hyttinen J, Miettinen S. Functional Outcome of Human Adipose Stem Cell Injections in Rat Anal Sphincter Acute Injury Model. *Stem Cells Transl Med* 2018; **7**: 295-304 [PMID: 29383878 DOI: 10.1002/sctm.17-0208]

66 **Nolte T**, Brander-Weber P, Dangler C, Deschl U, Elwell MR, Greaves P, Hailey R, Leach MW, Pandiri AR, Rogers A, Shackelford CC, Spencer A, Tanaka T, Ward JM. Nonproliferative and Proliferative Lesions of the Gastrointestinal Tract, Pancreas and Salivary Glands of the Rat and Mouse. *J Toxicol Pathol* 2016; **29**: 1S-125S [PMID: 26973378 DOI: 10.1293/tox.29.1S]

67 **Li X**, Guo X, Jin W, Lu J. Effects of electroacupuncture combined with stem cell transplantation on anal sphincter injury-induced faecal incontinence in a rat model. *Acupunct Med* 2018; **pii**: acupmed-2016-011262 [PMID: 29519860 DOI: 10.1136/acupmed-2016-011262]

68 **Frudinger A**, Kölle D, Schwaiger W, Pfeifer J, Paede J, Halligan S. Muscle-derived cell injection to treat anal incontinence due to obstetric trauma: pilot study with 1 year follow-up. *Gut* 2010; **59**: 55-61 [PMID: 19875391 DOI: 10.1136/gut.2009.181347]

69 **Frudinger A**, Pfeifer J, Paede J, Kolovetsiou-Kreiner V, Marksteiner R, Halligan S. Autologous skeletal-muscle-derived cell injection for anal incontinence due to obstetric trauma: a 5-year follow-up of an initial study of 10 patients. *Colorectal Dis* 2015; **17**: 794-801 [PMID: 25773013 DOI: 10.1111/codi.12947]

70 **Romaniszyn M**, Rozwadowska N, Nowak M, Malcher A, Kolanowski T, Walega P, Richter P, Kurpisz M. Successful implantation of autologous muscle-derived stem cells in treatment of faecal incontinence due to external sphincter rupture. *Int J Colorectal Dis* 2013; **28**: 1035-1036 [PMID: 23549961 DOI: 10.1007/s00384-013-1692-y]

71 **Romaniszyn M**, Rozwadowska N, Malcher A, Kolanowski T, Walega P, Kurpisz M. Implantation of autologous muscle-derived stem cells in treatment of fecal incontinence: results of an experimental pilot study. *Tech Coloproctol* 2015; **19**: 685-696 [PMID: 26266767 DOI: 10.1007/s10151-015-1351-0]

72 **Sarveazad A**, Newstead GL, Mirzaei R, Joghataei MT, Bakhtiari M, Babahajian A, Mahjoubi B. A new method for treating fecal incontinence by implanting stem cells derived from human adipose tissue: preliminary findings of a randomized double-blind clinical trial. *Stem Cell Res Ther* 2017; **8**: 40 [PMID: 28222801 DOI: 10.1186/s13287-017-0489-2]

73 **Boyer O**, Bridoux V, Giverne C, Bisson A, Koning E, Leroi AM, Chambon P, Déhayes J, Le Corre S, Jacquot S, Bastit D, Martinet J, Houivet E, Tuech JJ, Benichou J, Michot F; and the Study Group of Myoblast Therapy for Faecal Incontinence. Autologous Myoblasts for the Treatment of Fecal Incontinence: Results of a Phase 2 Randomized Placebo-controlled Study (MIAS). *Ann Surg* 2018; **267**: 443-450 [PMID: 28426476 DOI: 10.1097/SLA.0000000000002268]

74 **Park EJ**, Kang J, Baik SH. Treatment of faecal incontinence using allogeneic-adipose-derived mesenchymal stem cells: a study protocol for a pilot randomised controlled trial. *BMJ Open* 2016; **6**: e010450 [PMID: 26888731 DOI: 10.1136/bmjopen-2015-010450]

75 **Salcedo L**, Damaser M, Butler R, Jiang HH, Hull T, Zutshi M. Long-term effects on pressure and electromyography in a rat model of anal sphincter injury. *Dis Colon Rectum* 2010; **53**: 1209-1217 [PMID: 20628287 DOI: 10.1007/DCR.0b013e3181de7fe0]

76 **Bohl JL**, Zakhem E, Bitar KN. Successful Treatment of Passive Fecal Incontinence in an Animal Model Using Engineered Biosphincters: A 3-Month Follow-Up Study. *Stem Cells Transl Med* 2017; **6**: 1795-1802 [PMID: 28678378 DOI: 10.1002/sctm.16-0458]

77 **Resplande J**, Gholami SS, Graziottin TM, Rogers R, Lin CS, Leng W, Lue TF. Long-term effect of ovariectomy and simulated birth trauma on the lower urinary tract of female rats. *J Urol* 2002; **168**: 323-330 [PMID: 12050564 DOI: 10.1016/S0022-5347(05)64915-4]

78 **Sievert KD**, Bakircioglu ME, Tsai T, Nunes L, Lue TF. The effect of labor and/or ovariectomy on rodent continence mechanism--the neuronal changes. *World J Urol* 2004; **22**: 244-250 [PMID: 15365750 DOI: 10.1007/s00345-004-0444-6]

79 **Healy CF**, O'Herlihy C, O'Brien C, O'Connell PR, Jones JF. Experimental models of neuropathic fecal incontinence: an animal model of childbirth injury to the pudendal nerve and external anal sphincter. *Dis Colon Rectum* 2008; **51**: 1619-26; discussion 1626 [PMID: 18779998 DOI: 10.1007/s10350-008-9283-7]

80 **Ghadge SK**, Mühlstedt S, Ozcelik C, Bader M. SDF-1α as a therapeutic stem cell homing factor in myocardial infarction. *Pharmacol Ther* 2011; **129**: 97-108 [PMID: 20965212 DOI: 10.1016/j.pharmthera.2010.09.011]

81 **Bigalke C**, Luderer F, Wulf K, Storm T, Löbler M, Arbeiter D, Rau BM, Nizze H, Vollmar B, Schmitz KP, Klar E, Sternberg K. VEGF-releasing suture material for enhancement of vascularization: development, in vitro and in vivo study. *Acta Biomater* 2014; **10**: 5081-5089 [PMID: 25204522 DOI: 10.1016/j.actbio.2014.09.002]

82 **Yao J**, Korotkova T, Riboh J, Chong A, Chang J, Smith RL. Bioactive sutures for tendon repair: assessment of a method of delivering pluripotential embryonic cells. *J Hand Surg Am* 2008; **33**: 1558-1564 [PMID: 18984338 DOI: 10.1016/j.jhsa.2008.06.010]

83 **Horváthy DB**, Vácz G, Cselenyák A, Weszl M, Kiss L, Lacza Z. Albumin-coated bioactive suture for cell transplantation. *Surg Innov* 2013; **20**: 249-255 [PMID: 22717700 DOI: 10.1177/1553350612451353]

84 **Nauta AJ**, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007; **110**: 3499-3506 [PMID: 17664353 DOI: 10.1182/blood-2007-02-069716]

85 **Chen L**, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008; **3**: e1886 [PMID: 18382669 DOI: 10.1371/journal.pone.0001886]

86 **Németh K**, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009; **15**: 42-49 [PMID: 19098906 DOI: 10.1038/nm.1905]

87 **Riera del Moral L**, Largo C, Ramirez JR, Vega Clemente L, Fernández Heredero A, Riera de Cubas L, Garcia-Olmo D, Garcia-Arranz M. Potential of mesenchymal stem cell in stabilization of abdominal aortic aneurysm sac. *J Surg Res* 2015; **195**: 325-333 [PMID: 25592273 DOI: 10.1016/j.jss.2014.12.020]

88 **Røsland GV**, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, Mysliwietz J, Tonn JC, Goldbrunner R, Lønning PE, Bjerkvig R, Schichor C. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res* 2009; **69**: 5331-5339 [PMID: 19509230 DOI: 10.1158/0008-5472.CAN-08-4630]

89 **Garcia S**, Bernad A, Martín MC, Cigudosa JC, Garcia-Castro J, de la Fuente R. Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells. *Exp Cell Res* 2010; **316**: 1648-1650 [PMID: 20171963 DOI: 10.1016/j.yexcr.2010.02.016]

90 **Torsvik A**, Røsland GV, Svendsen A, Molven A, Immervoll H, McCormack E, Lønning PE, Primon M, Sobala E, Tonn JC, Goldbrunner R, Schichor C, Mysliwietz J, Lah TT, Motaln H, Knappskog S, Bjerkvig R. Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track - letter. *Cancer Res* 2010; **70**: 6393-6396 [PMID: 20631079 DOI: 10.1158/0008-5472.CAN-10-1305]

91 **Rhodes LV**, Muir SE, Elliott S, Guillot LM, Antoon JW, Penfornis P, Tilghman SL, Salvo VA, Fonseca JP, Lacey MR, Beckman BS, McLachlan JA, Rowan BG, Pochampally R, Burow ME. Adult human mesenchymal stem cells enhance breast tumorigenesis and promote hormone independence. *Breast Cancer Res Treat* 2010; **121**: 293-300 [PMID: 19597705 DOI: 10.1007/s10549-009-0458-2]

92 **Xu WT**, Bian ZY, Fan QM, Li G, Tang TT. Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis. *Cancer Lett* 2009; **281**: 32-41 [PMID: 19342158 DOI: 10.1016/j.canlet.2009.02.022]

93 **Cousin B**, Ravet E, Poglio S, De Toni F, Bertuzzi M, Lulka H, Touil I, André M, Grolleau JL, Péron JM, Chavoin JP, Bourin P, Pénicaud L, Casteilla L, Buscail L, Cordelier P. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. *PLoS One* 2009; **4**: e6278 [PMID: 19609435 DOI: 10.1371/journal.pone.0006278]

94 **Sun N**, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A* 2009; **106**: 15720-15725 [PMID: 19805220 DOI: 10.1073/pnas.0908450106]

95 **Khakoo AY**, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, Nguyen AT, Malide D, Combs CA, Hall G, Zhang J, Raffeld M, Rogers TB, Stetler-Stevenson W, Frank JA, Reitz M, Finkel T. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006; **203**: 1235-1247 [PMID: 16636132 DOI: 10.1084/jem.20051921]

96 **Klopp AH**, Gupta A, Spaeth E, Andreeff M, Marini F 3rd. Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? *Stem Cells* 2011; **29**: 11-19 [PMID: 21280155 DOI: 10.1002/stem.559]

97 **Ramdasi S**, Sarang S, Viswanathan C. Potential of Mesenchymal Stem Cell based application in Cancer. *Int J Hematol Oncol Stem Cell Res* 2015; **9**: 95-103 [PMID: 25922650]

98 **Chulpanova DS**, Kitaeva KV, Tazetdinova LG, James V, Rizvanov AA, Solovyeva VV. Application of Mesenchymal Stem Cells for Therapeutic Agent Delivery in Anti-tumor Treatment. *Front Pharmacol* 2018; **9**: 259 [PMID: 29615915 DOI: 10.3389/fphar.2018.00259]

99 **Kuriyan AE**, Albini TA, Townsend JH, Rodriguez M, Pandya HK, Leonard RE 2nd, Parrott MB, Rosenfeld PJ, Flynn HW Jr, Goldberg JL. Vision Loss after Intravitreal Injection of Autologous "Stem Cells" for AMD. *N Engl J Med* 2017; **376**: 1047-1053 [PMID: 28296617 DOI: 10.1056/NEJMoa1609583]

100 **Davies BM**, Smith J, Rikabi S, Wartolowska K, Morrey M, French A, MacLaren R, Williams D, Bure K, Pinedo-Villanueva R, Mathur A, Birchall M, Snyder E, Atala A, Reeve B, Brindley D. A quantitative, multi-national and multi-stakeholder assessment of barriers to the adoption of cell therapies. *J Tissue Eng* 2017; **8**: 2041731417724413 [PMID: 28835816 DOI: 10.1177/2041731417724413]

101 **Danielson J**, Karlbom U, Graf W, Wester T. Long-term outcome after free autogenous muscle transplantation for anal incontinence in children with anorectal malformations. *J Pediatr Surg* 2010; **45**: 2036-2040 [PMID: 20920725 DOI: 10.1016/j.jpedsurg.2010.06.009]

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**Table 1 Faecal Incontinence models employed in published preclinical studies and type of reparation system**

|  |
| --- |
| **Faecal Incontinence models in preclinical studies** |
| **Surgical injury** | **Crioinjury** | **Pudendal nerve crush** | **No injury** |
| SectionAnterior: 2Left lateral: 9[[43](#_ENREF_43)] Posterior subtotal: 3Proctoepisiotomy: 125% excision: 450% excision (IAS: 1) (both: 3)Total excision EAS: 1 | 2 | 1[[43](#_ENREF_43)] | 3 |
| **Type of reparations employed** |
| **Surgical repair** | **No repair** | **Substitution** | **Not applicable** |
| 6Randomized 2[[39](#_ENREF_39),[64](#_ENREF_64)]  | Crioinjury: 2Section: 9[[39](#_ENREF_39),[43](#_ENREF_43),[64](#_ENREF_64)]Excision: 7 | 2 | 3 |

Numbers indicate the number of published studies. EAS: External anal sphincter; IAS: Internal anal sphincter.

**Table 2 Kind of stem cells employed in published preclinical studies classified by origin organ and type of transplant**

|  |
| --- |
| **Kind of stem cells employed** |
| **Muscle progenitors** | **Bone marrow SCs** | **ASCs** | **Enteric neural** | **USCs** |
| Myoblasts: 6Muscle SCs: 9 | BM-MSCs: 10[[35](#_ENREF_35),[38](#_ENREF_38),[60](#_ENREF_60)]Mononuclear: 1[[60](#_ENREF_60)] | Aut: 1Xenog: 1 | Aut: 1Xenog: 1 | Xenog: 1[[38](#_ENREF_38)] |
| **Autologous/syngeneic** | **Allogeneic** | **Xenogeneic** |
| 11[[35](#_ENREF_35)] | 17[[35](#_ENREF_35),[38](#_ENREF_38)] | 3[[38](#_ENREF_38)] |

Numbers indicate the number of published studies. ASCs: Adipose-derived stem cells; USCs: Umbilical cord stem cells; BM-MSCs: Bone marrow derived mesenchymal stem cells.

**Table 3 Bioengineering strategies combined with stem cells and scaffolds employed as stem cells carriers to improve their function in published preclinical studies**

|  |
| --- |
| **Bioengineering models** |
| [[46](#_ENREF_46),[55](#_ENREF_55),[56](#_ENREF_56)] | Polycaprolactone beads |
| [[51](#_ENREF_51)] | IAS muscle cells + human ENPC + bilayer collagen and laminin hydrogel |
| [[57](#_ENREF_57)] | Polyethylene glycol-based hydrogel matrix scaffold |
| [[58](#_ENREF_58)] | Decellularized EAS |
| [[76](#_ENREF_76)] | IAS cells + enteric neural progenitor cells (biosphincter) |
| [[65](#_ENREF_65)] | Polyacrylamide hydrogel carrier (Bulkamid) |
| [[61](#_ENREF_61),[63](#_ENREF_63)] | Gelatin scaffold |

ENPC: Enteric neural progenitor cells; EAS: External anal sphincter; IAS: Internal anal sphincter.

**Table 4 Overview and concise review of different published studies related to Faecal Incontinence and stem cell therapy in animal models**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Animal** | ***N*** | **Randomized** | **Type of SC** | **Compared to** | **FI model** | **Repair?** | **Treatment** | **Effect measure** | **Follow up** | **Principal Results** | **Security concerns** |
| [[35](#_ENREF_35)] | Rats | 32 | No | AUT/ALLOG BM-MSCs | Sham injuryInjury + SSF | Surg section | Surg | Inj IE | Histology*In vitro* contractility | 30 d | ↑ muscular area ↑ Electric response and relaxing | No |
| [[36](#_ENREF_36)] | Rats | 15 | No | MDSC AUT | No injuryCrioinj/Crioinj+SCs | Crioinjury | No | Inj IE | Histology*In vitro* contractility | 7 d | SC Survive + myofibre differentiation ↑ contractility (NSS) | No |
| [[37](#_ENREF_37)] | Rats | ?? | No | Myoblast ALLOG | Subcutaneouslevator anithig muscle | No | No | Inj levator ani | Histology | ?? | SC survivorinjury necessary for myofibre formation | No |
| [[38](#_ENREF_38)] | Rab-bits | 31 | No | hUSCs SYNGBM-MSCs ALLOG | Culture mediumSaline | Section | No | Inj IE2 wk later | ClinicEMGHistology | 2 wk | BM-MSC: better continence ↑ act SS↑ muscle  | No |
| [[39](#_ENREF_39)] | Rats | 120 | Yes | MDSC ALLOG | Saline | Surg section EAS | Surg | Inj IE | Contractility | 13 wk | ↑ SS contractility 7/90d only repaired | No |
| [[40](#_ENREF_40)] | Rats | 4 | No | Myoblasts ALLOG | None | No | No | Inj IE | Histology | 10 d | SC survival and integration in sane host tissue | No |
| [[41](#_ENREF_41)] | Rabbits | 21 | No | MDSC AUT | Saline | Surg section EAS | No | Inj IE3 wk later | ClinicHistologyEMG+MAR | 2 mo6 mo (control) | ↑ continence since 4w Miotube + myofibre (4w), SC Survival,↓Cd3&cd34 cells, ↑ proliferate.↑SS MAR & EMG since 4w and grew | No |
| [[42](#_ENREF_42)] | Rats | 224 | No | BM-MSCs ALLOG local/systemic | PBS local/Syst | Surg section EAS | Surg | Inj IE/systemic | MollecularHistologyNeurophisiology | 21 d | Local: ↑ECM acute phase↑ fibers SS detected 24-48h (no later)↑ activity  | ↑ mortality nearly SSsystemic |
| [[43](#_ENREF_43)] | Rats | 70 | Yes | BM-MSCs ALLOGlocal/systemic | PBS local/Syst/Sham injuries | Surg sectionPNC | No | Inj IE/systemic | MAR + EMG | 10 d | IM/IV improve MAR, IV MAR non after PNCNo SC survivor | No |
| [[46](#_ENREF_46)] | Dogs | 10 | No | Myoblast AUT+ bioengineering | SC/nothing | Excision 25% AS | No | Inj IE3 mo later | CMAP/MAR Histology | 3 mo | ↑ MAR (non SS)Foreign body reaction | No |
| [[47](#_ENREF_47)] | Rats | 33 | No | MDSCs ALLOG | Sham control(9 *vs* 24 rats) | Surg Section | Surg | Inj IE | Migration lung-liverAS histology | 30 d | No migration | 2 benign local foci |
| [[48](#_ENREF_48)] | Rats | 45 | No | Myoblast SYNG | Uninjured Crioinj + PBS | Crioinjury | No | Inj IE | Histology/MAR | 2 mo (histo)6 mo (function) | Restitutio (60d), SC integrated↑ MAR 30d, SS from 60d | No |
| [[49](#_ENREF_49)] | Rats | 33 | Yes | MDSC ALLOG | PBS | Surg section (Proctoepisio) | Surg | Inj IE | MAR + EMGHistology | 4 wk | Improve SS EMG+MAR 2w not 4wNo differences sphincter thickness | No |
| [[50](#_ENREF_50)] | Rabbits | 12 | Yes | MDSC AUT | Saline | Surg section EAS | No | Inj IE3w later | MRI/MAR + EMG Histology | 4 wk | Labelled cells in MRI+areas, iron+ myofibre↑ ES MAR y EMG | No |
| [[51](#_ENREF_51)] | Rats | ?? | No | Neural EntericProgenitors XENOG | No injury/Crio/Crio+SCs | NO | No | BE: NPC + IAS cells + bilayer | Histology/EMG | 4 wk | ↑ neovascularizationnormal functioning | No |
| [[53](#_ENREF_53)] | Rats | 50 | Yes | BM-MSCs ALLOGlocal/systemic | SalineUninjured | Excision 25% AS | No | Inj IE/serial IV 24 h/3 wk later | MAR Histology (immunofluoresc) | 5 wk | -↑ P 10d MSCs, 5w MSC>Saline but no differences with uninjuredHistology: ↓gap, fibrosis, scar / Delayed 3w no efficacy. | No |
| [[54](#_ENREF_54)] | Rats | 40 | Yes | MDSC ALLOG | PBS | Surg section | Surg | Inj IE | Histology | 3 mo | No differences between groups. | No |
| [[55](#_ENREF_55)] | Dogs | 15 | No | Myobl AUT+ PCL beads | PBSUninjured | Excision 25% AS | No | Inj IE1m later | MARHistology | 3 mo | ↑ SS MAR (50% basal)SC survival + differentiation | No |
| [[56](#_ENREF_56)] | Dogs | 10 | Yes | Myoblast AUT (A) | (B) Myob aut + PCL beads with bFGF | Excision 25% AS | No | Inj IE1 mo later | MAR/CMAPHistology | 3 mo | ↑ SS MAR+CMAP B>ASC en 40% (A) vs 100% (B) | No |
| [[57](#_ENREF_57)] | Rats | 80+ 20 | Yes | MDSC ALLOG+hidrogel | NothingPBS-hydrogelCollagen/No injury | Surg Section | No | Inj IE | ContractilityHistology | 3 mo | ↑Contract and ↑ all F-U in SC-Hydrogel↑ SS Muscle SC-Hydrogel;↓ inflammation SC-Hydrogel and collagen | No |
| [[58](#_ENREF_58)] | Rab-bits | 16 | No | MDSC AUT | Only EAS scafold | Total EAS excision | No | EASsustitution | Histol (every 3 mo)EMG 2 yr | 2 yr | No inflammation-reject, improve SS 3-6mImprove EMG (no statistics provided) | No |
| [[59](#_ENREF_59)] | Rats | 58 | Yes | BM-MSC ALLOG+ electrostim | No treatmentElecrostimulation | Excision 50% | No | Inj IE+ electrostim | Histology/MAR | 4 wk | 4w, electrostimulation + 1 dose MSCs: ↑ muscle in injury area↑ resting P compared with other groups | No |
| [[60](#_ENREF_60)] | Rats | 32 | No | BM-MSCs ALLOGBM mononuclear | Sham SurgerySSF | Surg section | Surg | Inj IE | Histol/Morphometry/MAR *In vitro* contractility | 30 d | SC ↑ regen and SS contractility. No differences between SCSC survive 30d. | No |
| [[61](#_ENREF_61)] | Rats | 32 | Yes | BM-MSCs ALLO + SDF-1 (simult/deferred) | No treatmentSDF-1 | Excision 50% | No | Inj IE + SDF-1± gelatin scaffold | Histology/MAR | 4 wk | SDF-1 +/- SCs: ↑ resting P and %muscle and muscle organization and ↓ fibrosis (SS) | No |
| [[76](#_ENREF_76)] | Rabbits | 20 | No | Neural Enteric Progenitors AUT | No treatmentSham injury | Excision 50% IAS | No | Sustitution (biosphincter)6-8 wk later | Histology/MAR | 3 mo | Functional improvement since 1m, SS with othersRegeneration, neovascularization and innervation | No |
| [[63](#_ENREF_63)] | Rats | 56 | Yes | BM-MSCs ALLOG + SDF-1 (deferred) | No treatmentSDF-1 | Excision 50% | No | Inj IE + SDF-1± gelatin scaffold | HistologyMorphometryMAR Cytoquines | 8 wk | Plasmid +/-SCS: ↑ MAR, muscle organization Plasmid: ↑ muscle mass. SDF-1 sufficient for repairing without SC+/-scaffold | No |
| [[64](#_ENREF_64)] | Rats | 36 | Yes | ASCs SYNG | Conventional suture | Surg section | Yes/No | Inj IEbiosuture | Histology/MAR | 7 d | No functional differencesSC survivor and migration to injury | No |
| [[65](#_ENREF_65)] | Rats | 58 | Yes | Human ASCs | SSFBulkamid (hydrogel) | Surg section | Surg | Inj IE | MARmicro-CTHistology | 4 wk | Functional: ↑ SS ASCs and grew: no differences between carriersMorphology: no differences in muscle, >inflammation if ASCs, micro-CT correlation | No |
| [[67](#_ENREF_67)] | Rats | 60 | No | BM-MSCs ALLOG± electroacupunct | Sham injury Elecroacupunture SSF acupuncture | Surg section | No | Inj IV | Morphology | 14 d | SC+EA ↑vessels, fibroplasia and ↓ inflammation↑ muscle SS and homing growth factors (SS). Electroacupunct promotes homing. | No |

AUT: Autologous; ALLOG: Allogeneic; SYNG: Syngeneic; XENOG: Xenogeneic; SSF: Saline solution; Surg: Surgical; Inj: Injection; IE: Intrasphincteric; Crioinj: Cryoinjury; NSS: Non statistically significant; SS: Statistically significant; ??: Unknow; ECM: Extracellular matrix; AS: Anal sphincter; Proctoepisio: Proctoepisiotomy; NPC: Neural progenitor cells; Immunofluoresc: Immunofluorescence; P: Pressure; PCL: Polycaprolactone; F-U: Follow-up; Histol: Histology; Simult: Simultaneous; Electrostim: Electrostimulation; Electroacupunt: Electroacupuncture.

**Table 5 Overview of published clinical experience in stem cell therapy for Faecal Incontinence**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Study type** | ***N*** | **Stem****cell** | **Treat-ment** | **Compared** | **Other treatments** | **Effect measure** | **F-U** | **Principal results** |
| [[102](#_ENREF_102)] | Phase II RCT | 26 | AUT ASCs | Injection fistula | Fibrin glue | No | Subjective | 1 yr | Improvement 60% *vs* 23% |
| [[68](#_ENREF_68)] | Obser-vational | 10 | AUTMB | Injection EAS | No | Anal electrical estimulation 10 + 4 wk | ClinicalMARMorphology | 1 yr | ↓Wexner and episodes 1 yr, ↑QoL ↑ Voluntary P 1, 6 mo no at 12Morphology no changes  |
| [[69](#_ENREF_69)] | Obser-vational | = |  |   |   |   |   | 5 yr | ↓Wexner and episodes, ↑QoL ↑ P |
| [[70](#_ENREF_70)] | Case report | 1 | AUT MB | Injection EAS | No | No | Clinical MAR + EMG | 1 yr | Improved since 6 wk↑ P and EMG on scar area |
| [[71](#_ENREF_71)] | Obser-vational | 10 | AUTMB | Injection EAS | No | No | ClinicalMAR | 1 yr | MAR SS 18 wk Clinical: 66% 18 wk and 44.4% 1 yrEMG improvement all F-U |
| [[72](#_ENREF_72)] | RCT double-blind | 18 | ALLO ASCs | Injection EAS | PBS | Surgery | WexnerUSEMG | 2 mo | No differences on Wexner ↑ SS muscle area and EMG |
| [[73](#_ENREF_73)] | Phase II RCT | 24 | AUTMB | Injection EAS | Placebo | Biofeedback 15 d | Wexner, FIQL MAR, NPSUS, MRI | 1 yr | SS improve Wexner 1 yr, response 60%.Partial improvement FIQL 6-12 mo No morphologic differences at 12 moTransient placebo effect |

AUT: Autologous; ALLO: Allogeneic; RCT: Randomized controlled trial; MB: Myoblast; NPS: Neurophysiology; SS: Statistically significant; QoL: Quality of life; P: Pressure; F-U: Follow-up.