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

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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 for quality of life. Current management modalities are not ideal, and the development of new treatments is needed. Since 2008, stem cell therapies have been validated, 36 publications have appeared (29 in preclinical models and seven in clinical settings), and six registered clinical trials are currently 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. Cellular therapy has also been shown to stimulate...
the repair of both acute and subacute anal sphincter injuries, and some encouraging functional results have been obtained. Stem cells combined 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 yet has yielded some promising results, and appears to be safe. Further investigation in both animal models and clinical settings is necessary to drawing conclusions. Nevertheless, 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; Tissue engineering; Cell therapy; Stem cells

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**Core tip:** Faecal incontinence is very frequent and is associated with severe consequences for patients. Available treatment outcomes are not optimal, particularly in the long-term. Stem cells, with or without bioengineering, could improve these results, as demonstrated in other clinical settings. We present 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 of stem cells, 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], and Nelson found it in 2.2% of the general population and 47% of institutionalised individuals[2].

It is difficult to know its 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, which include the 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 of both with faecal incontinence[3]. Furthermore, it could also lead to job loss and is the second highest cause of institutionalisation. Studies focusing on the quality of life reflect significant repercussions in multiple components, such as physical[4,5] and mental[3]; generally, the greater the incontinence, the greater the deterioration it causes ($P < 0.01$)[5]. A Spanish study showed an independent association between quality of life and declining mental health (OR 2.088 and $P = 0.017$)[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, etc.) costs. Indirect costs are harder to calculate and account for more than half[5]. In a Seattle study, annual healthcare costs increased up to $2897 in 2005 according to multivariate analyses (pads, barriers or institutionalisation not included)[4]. In a Dutch study, global costs increased yearly by 2169€ for each patient[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 that depends 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 in almost 60% of patients is a sphincter lesion, with most of them being obstetric (30%-40%). Sphincter lesions during delivery range from 11%[8] to 26.9%[9], increase with every pregnancy, and cause incontinence in 76.8%-82.8% of patients[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]. 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]. 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 their quality of life). No failure predictive factors were found[12].
reasons for this decay are not well understood.

Stem cell (SC) therapy has been demonstrated to be safe and 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], mesenchymal stem cells (MSCs)[14-16] or adipose-derived stem cells (ASCs)[17-19]. For example, ASCs have been tried and have favourable outcomes in environments that are particularly unfavourable for wound healing, such as experimental colitis[20], sepsis[21], anal fistula[22-29], Crohn’s patients[30], experimental colonic[31,32], and tracheal[33] anastomoses.

Based on the published literature, as well as on our group’s experience with FI treatment and using ASCs in experimental and clinical settings (having conducted or participated in more than six clinical trials with autologous or allogeneic ASCs for digestive fistula), our aim was to review published literature related to stem cell therapy for FI, and currently ongoing clinical trials. To the best of our knowledge, there is only one review on this field from Gräs et al[34], which also includes 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” (http://www.clinicaltrials.gov) and the EU Clinical Trials Register (http://www.clinicaltrialsregister.eu) were searched using the same terms to detect ongoing registered clinical trials. Both searches were performed on April 1st 2018.

The high variability of FI models, the 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 in an animal model from Lorenzi et al[35] in 2008. From that point onwards, several articles have been published, mostly using animal models. However, only a few experiences with humans have gradually appeared since 2010.

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, that try to create a biocompatible and implantable EAS or IAS construct. Seven publications are on humans. Also, six registered clinical trials were found that 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). The types of animal used and the adjuvants employed to SCs are mentioned later.

The first publication was by Lorenzi et al[35] in 2008. They performed a left lateral selective sphincterotomy in male rats without specifying its length. The authors divided the animals into four groups of eight. 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 and saline injection (B). There were no relevant complications or exitus. After 30 d under histologic examination, a significant decrease in muscle tissue was observed at the site of repair, but morphometric analysis of groups C and D revealed a significantly greater muscle area than in group B (P < 0.05), but a significantly lower area than in group A. In functional 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] 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 × 10³ 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. In muscle strip in vitro contractility assays, cryoinjury significantly decreased contractility and MDSCs increased its amplitude without reaching statistical significance. Upon histological examination, they found labelled cells in all animals at the MDSC
injection site, confirming survival and tolerability (there were no immune responses in any animal), and also found differentiated muscle masses with variable orientations, suggesting partial myofiber (smooth and skeletal muscle) regeneration.

In 2009, Saihara et al.\cite{37} isolated allogeneic myoblasts from female F344 rats (at 1-4 wk), implanted them into nude mice, and evaluated myoblast evolution in 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 in subcutaneous fat at 3 wk, and became integrated into damaged muscles with myofiber formation at 4 wk. Nevertheless, in healthy muscle, myoblasts survive in smaller numbers, surround the muscle without integrating into it, and form myotubes but not myofibers. Therefore, injury stimulus may be fundamental to myofiber formation.

Aghaee-Afshar et al.\cite{38} published the first rabbit model in the same year, applying surgical damage (EAS lateral sectioning) without repair. Two weeks later, seven animals per group received human umbilical cord stem cells (uSCs, $10^4$), allogeneic rabbit BM-MSCs ($10^4$), culture medium or saline solution. These groups were also compared with three non-injured animals, all of which were evaluated before damage, before treatment and two weeks later. Clinical results: complete sphincter competence was found in four out of seven patients with BM-MSCs compared with two out of seven with uSCs, and partial competence in two out of seven with culture medium. On the electromyograph, there was a significant decrease in peaks per second

### Table 1  Faecal incontinence models employed in published preclinical studies and their types of reparations systems

| Surgical injury | Crioinjury | Pudendal nerve crush | No injury |
|-----------------|------------|----------------------|----------|
| Section         | 2          | 1\cite{43}           | 3        |
| Anterior: 2     |            |                      |          |
| Left lateral: 9 |            |                      |          |
| Posterior subtotal: 3 |            |                      |          |
| Proctoepisiotomy: 1 |            |                      |          |
| 25% excision: 4 |            |                      |          |
| 50% excision (IAS: 1) (both: 3) |            |                      |          |
| Total excision EAS: 1 |            |                      |          |

### Table 2  Origin of stem cells used in published preclinical studies, classified by organ origin and transplant type

| Kind of stem cells employed | Muscle progenitors | BM-MSCs: 10\cite{35,38,60} | ASCs: 9 | Enteric neural: 1\cite{38} | USCs: 1\cite{35} |
|-----------------------------|-------------------|-----------------------------|---------|---------------------------|------------------|
| Myoblasts: 6                |                   |                             | Aut: 1  | Aut: 1                    | Xenog: 1         |
| Muscle SCs: 9               |                   |                             | Xeno: 1 | Xenogeneic                |                  |
| Autologous/syngeneic        |                   |                             | Xeno: 1 |                          |                  |
| 11\cite{38}                |                   |                             |         |                          |                  |

### Table 3  Bioengineering strategies used with stem cells in published preclinical studies, and scaffolds employed as stem cell carriers to improve their function

| Bioengineering models       | Polycaprolactone beads | IAS muscle cells + human ENPC + bilayer collagen and laminin hydrogel | Polymethylene glycol-based hydrogel matrix scaffold | Decellularized EAS | IAS cells + enteric neural progenitor cells (biosphincter) | Polycrylamide hydrogel carrier (Bulkamid) | Gelatin scaffold |
|-----------------------------|------------------------|--------------------------------------------------------------------------|----------------------------------------------------|-------------------|----------------------------------------------------------|---------------------------------------------|-----------------|
| [46,55,56]                  |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [51]                       |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [57]                       |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [58]                       |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [76]                       |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [60]                       |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |
| [61,63]                    |                        |                                                                          |                                                    |                   |                                                          |                                                             |                 |

ENPC: Enteric neural progenitor cells; EAS: External anal sphincter; IAS: Internal anal sphincter.
after the injury, and a significant increase in BM-MSCs compared with pre-treatment values and controls; an insignificant increase appeared in uSCs, and no increase appeared in other groups. Both kinds of SCs were 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 as well as in all animals without SCs. Authors do not mention the percentage of implanted cells that survived, and do not confirm their differentiation into myofibers or their “normal” histology.

In 2010, White et al. published the first randomised study with 120 Sprague-Dawley virgin female rats. The authors performed a transection of EAS with a 7 mm incision (in this species, EAS is about 3-4 mm in longitudinal length). Animals were first randomly allocated to repair or no repair groups, and then each group received injected allogeneic pre-confluence myogenic stem cells (3.2 × 10⁶ on saline) or saline solution. If a repair was performed, a two-layer 5-0 braided polyglyactin interrupted suture (1 mm apart) was applied to the rectal mucosa, and EAS were approximated with two single interrupted stitches. Injections were applied under microscopic guidance in EAS ends (before repair, if scheduled). Animals were sacrificed at 1, 3 and 13 wk, and EAS contractility was studied in muscle strips in vitro. Seven days after injury, contractile function had severely declined, which was 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 short-term functional deterioration, 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. analysed the feasibility and safety of allogeneic rat myoblasts injected into the intact EAS of four 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 three and nine o’clock. Ten days later, the authors detected labelled cells within the EAS using immunofluorescence assays. To them, this demonstrated that myoblasts integrate into the host tissue.

Additionally in 2010, Kajbafzadeh et al. published a paper on rabbits. A surgical subtotal external sphincterotomy (9 mm longitudinal) was performed in the posterior part through an 8 mm longitudinal incision, with only the skin sutured. Three weeks later, autologous MDSCs (7 × 10⁷, nine animals) or saline buffer (12 animals) were injected into section borders. Sphincter electromyography (EMG) and manometry (ARM) were performed immediately before injury, as well as 14, 28, and 60 d after injection in three animals per group. Animals were sacrificed at every interval for histology studies. The three remaining animals from the 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, circular fibers around lesions in the control group 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 myofibers with variable disposition at 4 wk. At two and 4 wk, labelled cells were detected in all of the grafted sphincters, and there was less CD3+ cell infiltration at 4 wk (null at eight) with very few CD34+ cells appearing. These two last results confirm that findings cannot be explained by bone marrow-derived cell infiltration. A higher proliferative index was also identified with SCs. Upon functional examinations, injury promoted 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 in the control group with regard to functional and pathological parameters over time.

The following publication was issued by Pathi et al. in 2012, and first compared local and systemic SCs. They performed the same injury and repair as White et al. 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 seven and 21 postoperative days, using genetic sphincter expression by quantitative reverse transcription polymerase chain reaction (IL-10, IL-6, TGFβ1, TNFα, CPH-A, COX2, LOX) and with histology and neurophysiology results at 21 d. Upon functional evaluation at 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. Upon 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 fibers crossed the fibrinous area. Labelled cells were detected at 24 and 48 h, but not at seven 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 both TGF-β1 and LOX in the acute phase.

In 2013, Salcedo et al.\(^4\) published the first study that considered pudendal nerve injury using 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\(^4\) and pudendal nerve crush (comprising 30 seconds with a Castroviejo needle holder) as FI models. Animals were randomly assigned to: Surgical sphincterotomy (n = 20), pudendal nerve crush (n = 20), sham sphincterotomy (n = 10, five pressure) and sham pudendal nerve crush (n = 20, dissection only). Then, when they had previously demonstrated significant cytokine level changes (24 h after injury)\(^4\), they applied 2 × 10^6 labelled allogeneic BM-MSCs in PBS that were either injected into each of the four sphincter quadrants or intravenously (five animals for 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 EM-G after sphincterotomy, but not after pudendal nerve crush.

In 2013, Kang et al.\(^4\) published the first experiments 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. The dogs were randomly allocated to either the control group, or to the experimental group where they received an injection of porous polycaprolactone beads containing autologous myoblasts into the injury three months later (five dogs per group). The authors evaluated compound muscle action potentials (CMAPs) of the pudendal nerve, ARM, and histopathology three 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 of the control group, there was extensive damage to the muscle fibers with atrophy, cytoplasmic fibrosis and focal interstitial inflammatory cell infiltration. 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. These authors also insisted on the advantages of a large animal model to study effects (especially in vivo), mentioned the necessity of reinervation, and emphasised the study’s limitation due to the very low number of studied animals.

Also in 2013, Jacobs et al.\(^4\) published the first study with a safety concern. Here, 33 female virgin Sprague-Dawley rats received surgical anal sphincter transection and repair, after which 24 underwent the injection of 5.0 × 10^6 allogeneic MDSCs and nine served as 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 two local growth foci were noted in two animals receiving SCs. Further evaluations of them were consistent with a 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 on safety are needed, which could be focused locally since no migration appeared.

Furthermore, Bisson et al.\(^4\) published in 2013 a cryoinjury study on Fischer rats. The authors verified that the minimal lesion that caused sustainable deficiency was done from 90 degrees, which was repeated after a 24 h interval. Evaluations relied on both an electro-stimulated ARM as well as histology. The experimental groups were: Uninjured controls (n = 11), cryoinjured + PBS (n = 8), and cryoinjured + labelled syngeneic myoblasts injected with microscopic guidance. The novelties included the analysis of different doses and injection sites, and the first long-term follow-up (6 mo); three individual injections of 1 × 10^5 (n = 6), 1 × 10^5 (n = 8), or 1 × 10^6 (n = 6), two at the borders and the last within the lesion; alternatively, a single dose of 1 × 10^5 (n = 6) was injected into a unique site, within or opposite the lesion. Injections were well-tolerated. In the histology, EAS reconstitution

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was observed and SCs became integrated and differentiated into mature myofibers. 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 pressures 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.$^{[49]}$, which used Sprague-Dawley rodents. They first established normative EMG EAS parameters. A more radical procedure named proctoepliostomy, which involved an incision length of 5 mm 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 ($n = 24$, $5 \times 10^6$ 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, two 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 the experimental groups in EMG ($P < 0.01$) and ARM at 2 wk (the SC group recovered basal values), but there were no differences in EMG at 4 wk (both groups returned to baseline). Notably, 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.$^{[50]}$ published a study in 2014 focusing on SC homing and tracing, employing magnetic resonance imaging (MRI) for the first time in this field. They employed the Kazbafzadeh$^{[51]}$ model of FI in 12 rabbits. Animals were randomly assigned to receive either ultra-small superparamagnetic iron oxide (USPIO)-labelled $9 \times 10^7$ 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 before, 1 h after, and one, two, 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 ($\text{zone I}$) or absence ($\text{zone II}$) 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 a 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 myofibers staining positively for desmin as well as 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.

In the same year, Raghavan et al.$^{[52]}$ published the development and successful implantation of a bioengineered IAS by employing SCs in rats. Following their studies of bioengineered IAS since 2005$^{[53]}$, 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 that was 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, relevant neovascularisation, and normal contractility both in vitro and in vivo. Treated animals had also normal stooling.

The group of Salcedo and Zutshi from the Cleveland Clinic in Ohio, one of the most important in this field, published a randomized study in 2014$^{[54]}$. 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 ten to two 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 i.v. infusions ($n = 7$, evaluated at 10 d -5- and 5 wk -2-) 24 h later. Twenty-four hours 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 ($n = 5$). Rats were evaluated with ARM and immunofluorescence 10 d after treatment and at 5 wk. An additional group of ten rats underwent local (five rats) or i.v. (five 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 $5 \times 10^7$ labelled allogeneic BM-MSCs in PBS; in i.v. treatments, the same dosage was delivered daily for six consecutive days via the tail vein. Related to function, ten days after IM/IV MSC treatment, pressures were significantly increased compared with both the
et al. and labelled cells were detected, as well as some expressed myoblasts were differentiated in all animals because (group B, five dogs). ARM, pudendal nerve CMAPs (differentiation regulator) polycaprolactone beads bFGF-loaded (basic Fibroblast Growth Factor, a muscle differentiation regulator) polycaprolactone beads (group B, five dogs). ARM, pudendal nerve CMAPs and histology were evaluated at 3 mo. 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.

The last publication of 2014 is from Fitzwater et al.[54], and was a continuation of the White investigation using the same injury and repair procedures[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 × 10^7 at each site) and then euthanised at seven or 90 d (a half each period) for histological evaluation. The authors found sphincter disruption in 100% of the animals in both groups 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] 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] contributed with 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 1 mo after injury. In the first one[55], the authors compared a control group of sham surgery (only skin incision, n = 5) with ten injured dogs receiving polycaprolactone beads with PKH-26-labelled autologous myoblasts (n = 5) or PBS solution (n = 5) injected locally. 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, as well as some expressed smooth and skeletal muscle markers. In their second publication[56], they randomised ten injured dogs to receive either PKH-26-labeled autologous myoblasts (group A, five dogs) or autologous myoblasts and bFGF-loaded (basic Fibroblast Growth Factor, a muscle differentiation regulator) polycaprolactone beads (group B, five dogs). ARM, pudendal nerve CMAPs...
histological evaluation, no evidence of inflammation or rejection was observed and the transplanted EAS appeared normal; there were no morphological differences, but all immunohistochemical markers in the SC group revealed significant enhancement three and 6 mo after surgery ($P < 0.001$) without significant differences between 12 and 24 mo. In the functional evaluation of both groups, grafted EAS contracted in response to needle and electrical signals to both the muscle and pudendal nerve; more signals were always detected in group 1, but no statistical study about this issue was provided.

In 2016, Sun et al., also from Zutshi’s team, further expanded the concepts of delayed repair and SC homing. 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 three 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. A significant ($P = 0.03$) increase was found in MSC retention at the site of electrical stimulation with direct intramuscular injection (not in the other groups) compared to sham-stimulated animals. Finally, 16 SD rats underwent a ventral excision of 50% circumference of the anus and then randomly received (four animals each group): (1) no treatment; (2) daily electrical stimulation for 3 d; (3) 3 d stimulation follow by $10^6$ MSCs at the injury site the third day; and (4) 3 d stimulation with two injections of $10^7$ MSCs on 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, there was significantly more new muscle in the injured area four weeks after intervention, and there was also a significantly improved anal resting pressure in group 3 compared with all other groups.

Also in 2016, Mazzanti et al. (from Lorenzi’s group) published a study with 32 Lewis rats using Lorenzi's injury and primary repair models. There were four experimental groups: Sphincterotomy and primary repair 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., Six constructs were obtained from each animal and were supplemented with neural differentiation medium (Neurobasal-A). Each rabbit received four biosphincters (with two million smooth muscle cells and 800000 neural progenitors). Twenty female rabbits divided into 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 eight were 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 one and 3 mo after treatment; histology was also analysed. After the injury, all rabbits had significantly decreased basal tone and loss of both Recto-Anal Inhibitory Reflex (RAIR) and anal hygiene; these findings were sustained at 3 mo.

The same year, Sun et al. hypothesised that...
regenerating 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) 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 × 10^6 BM-MSCs 3 d later; and (4) plasmid and a gelatine scaffold mixed with BM-MSCs 3 d later. The 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, with no differences between groups receiving the plasmid. In morphology, all of the groups receiving the plasmid had significantly more organised muscle architecture than controls, with no 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 either MSCs or scaffold treatments.

In the first 3 mo of 2018, three publications have appeared. The first is from our research team, and is the pioneer study employing both autologous (syngenic) ASCs and biosutures for FI. First, anorectal normal anatomy was studied on Wistar and BDIX female rats. Then, an injury model consisting of a 1 cm extramucosal 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 (10^5 labelled ASCs) without repair; (2) biosuture repair (two sutures with 1.5 × 10^6 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 that were very heterogeneous in their 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 the interposed tissue, forming "conglomerates" with the 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 Kuismann et al 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 by sectioning both AS and anal mucosa, and then repaired them plane by plane with 6-0 poliglecaprone running sutures using magnifying loupes. Injections (at 30° and 330° on a superimposed clock face) were administered prior to perianal skin closure. They also tested whether ASC efficacy could be improved by adding a polycrylamide hydrogel carrier called Bulkamid. Female virgin rats were randomised into four groups (n = 14-15/group): hASCs (3 × 10^5) 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 the median resting and peak pressure were significantly higher at 2 and 4 wk in the 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 the 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, 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 suggested this for non-destructive morphometric analysis on the whole injured area.

The most recent publication is from Li et al, the pioneer evaluating electroacupuncture (with a galvanic stimulation) combined with SC therapy. The authors employed Zutshi's surgical injury with 60% 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 six consecutive days by inserting an acupuncture needle bilaterally 5 mm at the ST36 point and connecting them to a low-frequency electronic pulse instrument. BM-MSCs were administered with a single injection of 9.6 × 10^6 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-SCs and EA significantly increased capillary density, with the BM-SC + EA group having the highest values. Sarcomeric α-actinin expression was significantly higher at day 14 in groups 3–5 compared to 2 (injury only), 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-SC+EA group. Similar results were observed for SDF-1 and MCP-3 expression, suggesting the promotive effects of EA on the homing of BM-SCs. 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, substitution). The two most employed SCs include: 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 well-defined in the literature compared with MSCs; there is no consensus defining MDSCs and myoblasts as opposed to MSCs and ASCs, so the cellular products employed in publications could be more heterogeneous and could combine different cell lines. Thirteen studies randomly assigned treatments. Murine models are primarily employed (mainly for accessibility and lower cost: 21 studies), however bigger animal models have grown in the last years (looking for greater human similarity: five studies with rabbits and three with dogs have been published). More than one third of published studies have combined SCs and bioengineering with favourable results, and eight 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, and 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 (two local benign foci of growth in nearly 400 published injected animals) [47] and another with systemic (mortality increment associated in nearly 400 published injected animals) [42], possibly due to 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 [44,53,64] that find no differences using SCs or control products (placebo [54,44] or active [53]), and another one putting it in doubt [51]. The majority have confirmed SC survival in this potentially septic area, but some have not been able to find cells that retain SC labelling [42,53]. Most publications only perform short or at least medium-term follow-up (three–6 mo), with only one long-term follow-up (2 yr) published [60]. There are also many doubts concerning the mechanisms of action of SCs in this field.

We think that many more studies are needed to draw concrete 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 seven 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 (five studies, all autologous) and ASCs (one autologous and one 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. [61] 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 three improved (60%), compared to three out of 13 (23.08%) in the control group (fibrin glue) who did not improve [24]. The evaluation was purely subjective, and the study was not designed to accomplish this objective. These results should therefore be evaluated with caution.

The first specific publication was the observational study from Frudinger et al. [68]. The authors injected autologous myoblasts into the EAS from ten female patients with non-operated anterior lesions that were 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 $20.16 \times 10^6$ 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 units), daily defecations (0.4), and incontinence episodes per week (8) at the one year follow-up. Related to function, voluntary pressure grew significantly at one and 6 mo, but later decayed to basal values at 12 mo; maximal and median resting pressure also significantly decreased (7 and 6mmHg respectively) between six and 12 mo.

| Table 5 | Stem cell therapy for faecal incontinence | WJSC | www.wjgnet.com | July 26, 2018 | Volume 10 | Issue 7 |
| Ref. | Animal | N  | Randomized | Type of SC | Compared to | FI model | Repair? | Treatment | Effect measure | Follow up | Principal Results | Security concerns |
|------|--------|----|------------|------------|-------------|----------|---------|-----------|---------------|-----------|------------------|------------------|
| [35] | Rats   | 32 | No         | AUT/ALLOG BM-MSCs | Sham injury Injury + SSF | Surg section | Surg | Inj IE | Histology In vitro contractility | 30 d | ↑ muscular area ↑ Electric response and relaxing | No |
| [36] | Rats   | 15 | No         | MDSC AUT | No injury Crioinjury + Crioinjury + 5Cs | Crioinjury | No | Inj IE | Histology In vitro contractility | 7 d | SC survive + myofibre differentiation ↑ contractility (NSS) | No |
| [37] | Rats   | ?? | No         | Myoblast ALLOG | Subcutaneous levator ani thig muscle | No | No | Inj levator ani | Histology | ?? | SC survivor injury necessary for myofibre formation | No |
| [38] | Rab-bits | 31 | No         | hUSCs SYNG BM-MSCs ALLOG | Culture medium | Section | No | Inj IE 2 wk later | Clinic EMG Histology | 2 wk | BM-MSC: better continence ↑ act SS ↑ muscle | No |
| [39] | Rats   | 120 | Yes        | MDSC ALLOG | Saline | Surg section EAS | Surg | Inj IE | Contractility | 13 wk | ↑ SS contractility 7/90 d only repaired | No |
| [40] | Rats   | 4  | No         | Myoblasts ALLOG | None | No | No | Inj IE | Histology | 10 d | SC survival and integration in saine host tissue | No |
| [41] | Rabbits | 21 | No         | MDSC AUT | Saline | Surg section EAS | No | Inj IE 3 wk later | Clinic Histology EMG + MAR | 2 mo 6 mo (control) | ↑ continence since 4w Myotube + myofibre (4wk), SC Survival, ↓ Cd3 and cd34 cells, ↑ proliferate ↑ SS MAR and EMG since 4wk and grew | No |
| [42] | Rats   | 224 | No         | BM-MSCs ALLOG local/systemic | PBS local/Syst | Surg section EAS | Surg | Inj IE/systemic | Molecular Histology Neurophysiology | 21 d | Local: ↑ECM acute phase ↑ fibers SS detected 24-48 h (no laser) ↑ activity ↑ mobility nearly SS systemic | No |
| [43] | Rats   | 70 | Yes        | BM-MSCs ALLOG local/systemic | PBS local/Syst/ Sham injuries | Surg section | PNC | No | Inj IE/systemic | MAR + EMG | IM/IV improve MAR, IV MAR non after PNC No SC survivor | No |
| [46] | Dogs   | 10 | No         | Myoblast AUT + bioengineering | SC/nothing | Excision 25% AS | No | Inj IE 3 mo later | CMAP/MAR Histology | 3 mo | ↑ MAR (non SS) Foreign body reaction | No |
| [47] | Rats   | 33 | No         | MDSCs ALLOG | Sham control (9 vs 24 rats) | Surg section | Surg | Inj IE | Migration lung-liver AS histology | 30 d | No migration 2 benign local foci | No |
| Ref  | Species | No. | Sample Source | Treatment | Repair | Biopsy | Repair Time | Other Comments |
|------|---------|-----|----------------|-----------|-------|---------|-------------|----------------|
| 48   | Rats    | 45  | Myoblast SYNG  | Uninjured crioinj + PBS | No inj IE | Histology/MAR | 2 mo histo, 6 mo function | Restitutio (60 d), SC integrated, ↑ MAR 30 d, SS from 60 d |
| 49   | Rats    | 33  | MDSC ALLOG     | PBS        | Surg section (Proctoepisio) | Surg inj IE | MAR + EMG Histology | 4 wk Improve SS EMG + MAR 2wk not 4wk, No differences in sphincter thickness |
| 50   | Rabbits| 12  | MDSC AUT       | Saline     | Surg section EAS | No inj IE | MRI/MAR + EMG Histology | 4 wk Labelled cells in MRI + areas, iron + myofibre ↑ ES MAR y EMG |
| 51   | Rats    | ?7  | Neural enteric progenitors XENOG | No injury/Crio / Crio + SCs | NO | BE: NPC + IAS cells + bilayer | Histology/EMG | ↑ neovascularization normal functioning |
| 52   | Rats    | 50  | BM-MSCs ALLOG local/systemic | Uninjured | Excision 25% AS | Inj IE/serial IV 24 h/3 wk later | MAR Histology (immunofluoresc) | ↑ P 10d MSCs, 5wk MSC > Saline but no differences with uninjured Histology: ↓ gap, fibrosis, scar/ Delayed 3wk no efficacy |
| 53   | Rats    | 40  | MDSC ALLOG     | PBS        | Surg section Surg | Inj IE | Histology | 3 mo No differences between groups |
| 54   | Dogs    | 15  | Myoblast AUT + PCL beads | PBS        | Uninjured | Excision 25% AS | No inj IE | MAR Histology | 3 mo ↑ SS MAR (50% basal), SC survival differentiation |
| 55   | Dogs    | 10  | Myoblast AUT (A) | PBS        | Uninjured | Excision 25% AS | No inj IE | MAR/CMAP Histology | 3 mo ↑ SS MAR + CMAP B > A SC en 40% (A) vs 100% (B) |
| 56   | Rats    | 80+20 | MDSC ALLOG + hidrogel | PBS-hydrogel | Surg Section | No injury | Inj IE | Contractility Histology | 3 mo ↑Contract and ↑ all F-U in SC-Hydrogel, ↑ SS Muscle SC-Hydrogel, ↓ inflammation SC-Hydrogel and collagen |
| Study ID | Specimen | No/Yes | Cell Type | Treatment | Behavioral | Histology | Substance | Outcome |
|----------|-----------|--------|-----------|-----------|------------|-----------|-----------|---------|
| [58]     | Rab-bits 16 | No     | MDSC AUT  | Only EAS scaffold | Total EAS excision | No EAS substitution | Histol (every 3 mo) EMG 2 yr | No inflammation-reject, improve SS 3-6mo Improve EMG (no statistics provided) |
| [59]     | Rats 58    | Yes    | BM-MSC ALLOG + electrostim | No treatment Electrostimulation | Excision 50% | No Inj IE + electrostim | Histology/MAR | 4 wk 4wk, electrostimulation + 1 dose MSCs: ↑ muscle in injury area ↑ resting P compared with other groups |
| [60]     | Rats 32    | No     | BM-MSCs ALLOG BM mononuclear | Sham surgery | Surg section | Surg | Inj IE | Histol/ morphometry/ MAR In vitro contractility | 30 d SC ↑ regeneration and SS contractility No differences between SC SC survive 30 d |
| [61]     | Rats 32    | No     | BM-MSCs ALLOG + SDF-1 (simult/ deferred) | No treatment SDF-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) |
| [62]     | Rabbits 20 | No     | Neural enteric Progenitors AUT | No treatment | Sham injury | Excision 50% IAS | Sustitution (biosphincter) 6-8 wk later | Histology/MAR | 3 mo Functional improvement since 1mo, SS with others Regeneration, neovascularization and innervation |
| [63]     | Rats 56    | Yes    | BM-MSCs ALLOG + SDF-1 (deferred) | No treatment SDF-1 | Excision 50% | No Inj IE + SDF-1 ± gelatin scaffold | Histology Morphometry MAR Cytoquines | 8 wk Plasmid +/- SCs: ↑ MAR, muscle organization Plasmid: ↑ muscle mass SDF-1 sufficient for repairing without SC+ / -scaffold |
| [64]     | Rats 36    | Yes    | ASCs SYNG | Conventional suture | Surg section | Yes/No | Inj IE biosuture | Histology/MAR | 7 d No functional differences SC survivor and migration to injury |
with a functional correlation. Five years later, a long-term evaluation was published\(^6^9\) that analyzed defecatory diaries, blood analyses, quality of life and function annually. No adverse events or changes in blood analyses appeared. Wexner, resting and voluntary contraction pressures, as well as the overall and sub-measures of quality of life, improved significantly \((P < 0.001)\) for the entire evaluated period. Reduced defecation frequency and the number of FI episodes also persisted for five years.

Romaniszyn et al.\(^7^0\) initially published, as a proof-of concept, a case of autologous myoblast implantation in EAS. Cells were obtained from the quadriceps, and the patient had a traumatic AS rupture refractory to both sphincteroplasty and biofeedback; an 8-10 mm scar on both AS persisted, and the Faecal Incontinence Severity Index (FISI) score was 20 points. Here, \(6 \times 10^8\) myoblasts were transplanted under ultrasonographic guidance and 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 activity in the EMG started to register on the scar area, where there was no activity before implantation. These results motivated them to perform a prospective study on ten patients that was published in 2015\(^7^1\). They included patients with FI of different origins with a Wexner (CCI) > ten, as well as low pressures with preserved reflex and innervation. In addition, they excluded patients with Wexner = 20, EAS defects > 90° and denervation. They implanted \(3 \times 10^8\) myoblasts distributed into 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, and if there was no defect, 3 mL was distributed around the EAS. No muscle biopsies or implantation procedures generated complications. Regarding ARM, no changes appeared at 6 wk, but values gradually increased at 12 and 18 wk (significantly at 18). After 18 wk, significant subjective improvement was obtained in six patients (66.7%), and all patients improved in ARM, five very significantly (55.6%). Upon EMG evaluation, improvement was found in all visits, with the highest values at 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

| Ref | Rats | Yes/No | Human ASCs | SSF | Surg section | Surg | Inj IE | MAR | 4 wk | Functional | Differences | Morphology | Inflammation | ASCs | homing factors | No |
|-----|------|--------|-------------|-----|---------------|-----|--------|-----|------|------------|-------------|------------|--------------|------|----------------|-----|
| 65  | Rats | Yes    | Human ASCs | SSF | Surg section  | Surg | Inj IE | MAR | 4 wk | Functional | Differences | Morphology | Inflammation | ASCs | homing factors | No |
| 66  | Rats | No     | BM-MSCs ALLOG ± electrouacupuncture | Surg section | No | Inj IV | Morphology | 14 d | SC+EA ↑ vesicles, fibroplasia and ↓ inflammation ↑ muscle SS and homing growth factors | (SS). Electroacupuncture promotes homing | No |
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.²². Twenty patients were randomised, but 18 were analysed (one exclusion due to cancer diagnosis before treatment, and one lost in follow-up) in two groups: both received a non-overlapping sphincteroplasty with 3-0 PDS and then received either 6 × 10⁶ ASCs (nine patients, one-half injected into each end of the muscle) or PBS (nine patients). Two months later, the CCI score, endorectal sonography, and EMG were recorded. No adverse events related to SC were detected. Both groups improved their Wexner scores without differences. In echography and EMG, the ratio of the area occupied by the muscle to the total lesion area showed a significant (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 surgeries that replace fibrous tissue with muscle. The trial was registered at the Iranian Registry of Clinical Trials with the code IRCT2016022826316N2.

Finally, this year, Boyer et al.²³ published a phase II randomised placebo-controlled study using autologous myoblasts. 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 receiving placebo. Eight injections of 100 ± 20 × 10⁶ SCs or placebo were made into both the remnant EAS and circumferentially as an outpatient procedure under echography guidance. A seven-day 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 one year. The follow-up consisted of visits at six and 12 mo, as well as the completion of CCI (primary endpoint), FIQL scores, ARM, perineal electrophysiological tests, anal sonography, and MRI. Related to the primary endpoint, the median CCI at 6 mo 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 the SC group (6.5 vs 15, P = 0.006), while the effect was lost in the placebo group (14 vs 15, P = 0.35), with a higher response rate observed 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 six- and 12-mo, and one and two of its components significantly ameliorated in the therapeutic arm at six and 12 mo, respectively.

Table 5  Overview of published clinical experience in stem cell therapy for faecal incontinence

| Ref. | Study type | N | Stem cell | Treatment | Compared | Other treatments | Effect measure | F-U | Principal results |
|------|------------|---|-----------|-----------|----------|-----------------|---------------|-----|------------------|
| [102] | Phase II RCT | 26 | AUT ASCs | Injection EAS | MB | No | Subjective | 1 yr | Improvement 60% vs 23% |
| [68] | Observational | 10 | AUT MB | Injection EAS | No | Anal electrical stimulation 10 + 4 wk | Clinical MAR Morphology | 1 yr | ↓ Wexner and episodes 1 yr, ↑QoL |
| [69] | Observational | 1 | AUT MB | Injection EAS | No | No differences on Wexner Observer Phase 24 | Subjective | 1 yr | ↓ Voluntary P 1, 6 mo no at 12 Morphology no changes |
| [70] | Case report | 1 | AUT MB | Injection EAS | No | No | Clinical MAR + EMG | 1 yr | Improved since 6 wk |
| [71] | Observational | 10 | AUT MB | Injection EAS | No | No | Clinical MAR | 1 yr | ↑ P and EMG on scar area |
| [72] | RCT double-blind | 18 | ALLO ASCs | Injection EAS | PBS | Surgery | Wexner US EMG | 2 mo | MAR SS 18 wk Clinical: 66% 18 wk and 44.4% 1 yr EMG improvement all F-U |
| [73] | Phase II RCT | 24 | AUT MB | Injection EAS | Placebo | Biodegradation 15 d | Wexner, FIQL MAR, NPS US, MRI | 1 yr | SS improve wexner 1 yr, response 60% |

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.
No change was evident for either arm on sonography, MRI or electrophysiological tests at 12 mo. No relevant adverse events were identified relatable to treatment. Therefore, SCs have shown tolerance, safety, and clinical benefits 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, the 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 ten patients from one study have surpassed a long-term evaluation[69]; the habitual follow-up is one year. More randomised and comparative studies, as well as long-term evaluations, are needed to draw conclusions about efficacy.

### ONGOING CLINICAL TRIALS

According to both the United States National Institutes of Health worldwide clinical trials registry (accessible from www.clinicaltrials.gov) and the EU Clinical Trials Register (www.clinicaltrialsregister.eu) on 1st April 2018, there were six registered clinical trials about stem cell therapy for FI. Surprisingly, there are no new records since previously performed search one year earlier, which is unusual for SC therapies since they are so extensively studied. We will describe them briefly here:

**NCT02292628**

A Spanish phase I/II triple-blinded randomised trial comparing autologous injected ASCs (4 × 10^7) with placebo in 16 patients.

**Inclusion:** A unique IAS defect and/or EAS (≤100°). 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 primary is FI Score at 24 wk, and the secondary is clinical assessment at 12 wk; maximum daily dry intervals (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 in South Korea focused on the security of local autologous ASCs (the registry does not specify the dose or implantation method).

**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 the secondary consists 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 and with two phases. Safety study: a dose escalation study: three groups of three patients receive 3 × 10^7, 6 × 10^7 or 9 × 10^7 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 six patients in each group. Employs randomised, open-label and single-blind design. Clinical assessment and follow-up are identical to the 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], but the recruitment status is “unknown”. The 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 it analyzes the injection of autologous muscle fibres and not SCs. However, a small part of the fibres is used for analysing MDSCs number and, therein, the regenerative potential of the sample.

**Eligible patients:** Patients with CCI ≥ 9 or affected quality of life after 3 mo with pelvic floor muscle training.

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

**Actual situation:** The recruitment status is "unknown", the completion date has passed, and the data has not been actualised within the past two years.

**NCT02687672**
A phase I/II trial in Jordan that is unrelated to FI. 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 over five years, and includes FI evaluation with a questionnaire as a secondary outcome. Currently active but non-recruiting, and the estimated completion is in December 2021.

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

**DISCUSSION**
FI is a frequent, chronic and highly limiting condition that mainly affects quality of life and has 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 eight excisions (from 25% to 100% of the sphincter complex).

In section models, Lorenzi et al. described a left lateral selective sphincterotomy without specifying the length. Zutshi’s model consisted of “a precise 3-4 mm incision”, which might not cause a total sphincter section. White et al. 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 1 cm in length, one of the most extensive sections. Going further, Lane et al. performed a more aggressive injury defined as a “proctoeisisotomy”, but did not describe the technical details or extension. Similar or minor modified procedures have been employed by Mazzanti et al., Salcedo et al., Pathi et al., Fitzwater, Montoya, Kuismanan, and Li.

A Salcedo publication in 2010 found that rats receiving Zutshi’s injury without repair or treatment presented anal pressure recovery 14 d later, comparable to twenty-one in rats. Other FI models include surgical (23 publications), including 15 sections and eight excisions (from 25% to 100% of the sphincter complex).

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simulate labour, an episiotomy and balloon extraction was performed. Later, Healy et al[70] published a model for FI using two intrapelvic, retromesenteric balloons (six Fr urinary catheters) placed through a 3 cm laparotomy for one hour. More studies on simulated childbirth models are possibly needed.

On the other hand, it is known in clinical settings 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 nine delayed treatments (repairing or injections delayed 2[38], 3[41,46,60,53] or 4 wk[35,66] and substitutions delayed 6-8 wk[76] or 6 mo[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] (mucosal tears, faecal contamination, etc.) that could compromise SCs survivorship or effects. However, there are also cytokines that are fundamental for SC homing and activation, as has been demonstrated for acute myocardial infarction with the SDF-1 factor[86]. In this field, Salcedo et al[45] made some relevant contributions; they studied Stromal derived factor-1 (SDF-1 or CXCL12) and monocyte chemotactic protein-3 (MCP-3), known signals that force homing of BM-MSCs to ischaemic tissue, and their 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]. Therefore, in chronic conditions, these molecules will have normal values and thus could make it difficult for SCs to act. To increase these factors, SCs could be transplanted with plasmids or the local production could be stimulated using surgical injury or electrical stimulation, such as in the paper by Sun[59]. The previously mentioned publications[61,63] open up an interesting new research field; the combination of SCs or their vehicles (for example sutures with Vascular Endothelial Growing Factor[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] could be useful for depositing SCs at the focus of the injury. Other authors have made different modifications to biosutures: Yao et al[85] added poly-L-lysine and fibronectin to improve cell adherence, and Horváthy et al[85] observed better BM-MSC adherence if the 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 × 10⁰ ASCs, SCs were found to form “clusters” both over the suture and in culture medium, and remained adhered after biosuture usage[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 the muscle layer with consequent cellular loss[64]. Injected doses have been very variable in the 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 will be very interesting in the future.

Related to the mechanism of SC actions, 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 phases, 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 likely base their role much more on immunomodulation, as well as on anti-inflammatory and angiogenic capabilities. There is growing evidence of the immunomodulation capability of MSCs, which is thought to be largely based on inhibition of T cell and B cell proliferation and dendritic cell maturation[64], as well as on the secretion of a large number of cytokines and growth factors[86]. Németh et al[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 shift from acute to chronic inflammation in the presence of ASCs (neutrophil descent and macrophage increment) after tracheal anastomosis[83], and Riera observed less acute and chronic inflammation during 3 mo, with the increasing fibrosis of the aneurysm sac in pigs[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 also be applied for another mechanism like 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 into an appropriate delivery system. Finally, we also need to 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 that are cultured for a long time may develop malignant changes and even promote tumours in mice[68]. However,
subsequent publications, including those from the same authors, attributed those findings to tumour cell cross-contamination[86,90]. Furthermore, other studies did not detect tumourigenesis 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 in the microenvironment, immunomodulation)[91,92] and anti-tumour (apoptosis, proliferation inhibition)[93,94] properties. This relationship depends on a lot of factors, including the type of MSCs, 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 of these effects is time. When MSCs are administered with an existing tumour, a suppressive effect has been observed[95], but in some studies with co-administration of SCs and tumour cells, tumour growth was higher compared to tumour cells alone[96]. These complex interactions have been studied by several authors and reviewed by Ramdasi et al[97].

Tropism to tumours has been exploited to treat tumours in experimental models, as reviewed by Chulpanova et al[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, leaving all with complete blindness (although the ASCs were mixed with blood plasma and large numbers of platelets)[99]. In conclusion, cumulated experience seems to support the oncogenic safety of SCs, but more studies and long-term follow-ups are needed to definitively exclude 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 questions remaining about SCs survival, effect, long-term results, efficacy on chronic conditions, etc. In human research, there is one unrelated study[24], six studies involving 55 patients receiving SCs with promising results[58-73] and six ongoing clinical trials. More highly rigorous investigations (related to SC type, dosage, delivery system, adjuvant factors, and safety) are needed before SC therapy for FI becomes a clinical reality.

Related to economy, 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 therapy adoption[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. If a cure could be achieved, direct and indirect costs mentioned before could disappear, and 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], approximated costs in Spain are the following: 1500-2500E (1727.8 to 2879.73 USD) for closed system SVF, 2800-4000E (3225.48-4607.83$) for 40×10⁶ autologous ASCs and 3500-5000E (4032.88-5761.26$) 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 first years). The first allogeneic ASC medicine product for fistula marketing is expected between 2018 and 2019, so we will be able to know the real costs of large-scale production. Moreover, some publications have reported acceptable results with free autologous muscle grafts in FI in children[101] (grafts also contain 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 need to be improved, so alternative treatments are therefore 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, healing biology, and bioengineering principles is needed before regenerative medicine for FI can become really implemented.

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