Regenerative medicine as a therapeutic option for fecal incontinence: a systematic review of preclinical and clinical studies

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BACKGROUND: Fecal incontinence is the uncontrollable loss of stool and has a prevalence of around 7-15%. This condition has serious implications for patients' quality of life. Current treatment options show unsatisfactory results. A novel treatment option is therefore needed.

OBJECTIVE: This systematic review aims to perform a quality assessment and to give a critical overview of the current research available on regenerative medicine as a treatment for fecal incontinence.

STUDY DESIGN: A systematic search strategy was applied in PubMed, Cochrane Library, EMBASE, MEDLINE, Web of Science, and Cinahl from inception until March of 2018. Studies were found relevant when the animals or patients in the studied group had objectively determined or induced fecal incontinence, and the intervention must have used any kind of cells, stem cells, or biocompatible material, with or without the use of trophic factors. Studies were screened on title and consecutively on abstract for relevance by 2 independent investigators. The risk of bias of preclinical studies was assessed using the SYstematic Review Centre for Laboratory animal Experimentation risk of bias tool for animal studies, and for clinical studies the Cochrane risk of bias tool for randomized trials was used.

RESULTS: In all, 34 preclinical studies and 5 clinical studies were included. Animal species, type of anal sphincter injury, intervention, and outcome parameters were heterogenous. Therefore, a meta-analysis could not be performed. The overall risk of bias of the included studies was high.

CONCLUSION: The efficacy of regenerative medicine to treat fecal incontinence could not be determined due to the high risk of bias and heterogenicity of the available preclinical and clinical studies. The findings of this systematic review may result in improved study design of future studies, which could help the translation of regenerative medicine to the clinic as an alternative to current treatments for fecal incontinence.

Key words: animal models, cell therapy, fecal incontinence, human studies, regenerative medicine, scaffolds, tissue engineering, trophic factors

Introduction

Fecal incontinence (FI) is the uncontrollable and involuntary loss of stool. This condition has serious implications for patients' quality of life and social activities. The prevalence of FI is estimated to be around 7−15%, but it is widely underreported because of its embarrassing character.[1]

Fecal continence is regulated by the rectum, the anal sphincter complex, and the puborectal muscle. This review focuses on FI caused by a damaged or dysfunctional anal sphincter complex. This complex consists of the internal anal sphincter (IAS) and the external anal sphincter (EAS). The IAS is an involuntary circular smooth muscle that is mainly responsible for the anal pressure at rest. The EAS is part of the levator ani muscle and consists of striated muscle fibers that are under both involuntary and voluntary control.[2]

Obstetric injury is a major cause of anal sphincter damage in women, with >35% of primiparous women experiencing some degree of injury to the EAS.[3] Conventional, noninvasive treatments for FI include diet and medication to manage stool consistency, and also biofeedback. Biofeedback refers to cognitively retraining the pelvic floor and abdominal wall muscles to improve patients' voluntary control of a biological response. Increasing the pressure in and around the anal sphincter by injecting bulking agents can also help to relieve FI.[4] Recently introduced surgical options are graciloplasty,[5,6] the artificial anal sphincter,[7] and sacral nerve stimulation.[8] Unfortunately, these treatments show unsatisfactory results. A Cochrane review on perianal injectable bulking agents showed no long-term evidence on outcomes.[4] Surgical treatments are associated with morbidity, high failure rates, and perioperative and long-term complications.[9−12] A systematic review concluded that the continence of patients with a functional anal sphincter device decreased over time.[13] Sacral nerve stimulation has a long-term success rate of only 53% and comes with adverse effects in 15% of patients.[8] A novel treatment option for FI is therefore needed.

In recent decades, stem cells have been applied to treat FI in preclinical and clinical studies. Local injection of stem

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AJOG at a Glance

Why was this study conducted?

Our objective was to perform a systematic review of the available preclinical and clinical studies in regenerative medicine as a treatment for fecal incontinence.

Key findings

Available research has a high risk of bias and is heterogeneous. Therefore, the efficacy of regenerative medicine to treat fecal incontinence cannot be determined.

What does this add to what is known?

This is the first systematic review concerning regenerative medicine for fecal incontinence. It takes into account the quality of available preclinical and clinical studies.

cells or other cells, scaffolds, and trophic factors can be added for a better, more durable effect. The use of scaffolds alone, or in combination with cells and/or growth factors, is called "tissue engineering." Tissue-engineered constructs are developed in vitro and can be used to replace the injured sphincter. This emerging field of research involving injections of cells and/or tissue-engineered constructs to promote regeneration is called "regenerative medicine" (RM).

Since RM promises to be a revolutionary new treatment for FI, the number of studies is increasing. However, preclinical and clinical studies are currently being performed in parallel and there is no consensus about the best RM strategy to treat FI. Therefore, the purpose of this systematic review is to perform a quality assessment and to give a critical overview of the current research available in preclinical and clinical studies using RM as a treatment for FI.

Materials and Methods

Literature search

To identify all available studies on RM for the treatment of FI published and indexed up to March 17, 2018, a systematic search strategy was applied in PubMed, Cochrane Library, EMBASE, Medline, Web of Science, and Cinahl (Table 1). This strategy combined preclinical (ie, animal) and clinical (ie, human) studies with a RM search component containing synonyms for RM and tissue engineering related terms with a customized search component for FI or anal sphincter repair. Medical Subject Headings (MeSH) terms and Emtree terms were used in PubMed and EMBASE, respectively, together with separate words or word combinations in title or abstract. In addition, retrieved reviews were screened for primary studies not found in the search strategy.

Study selection

Automatic deduplication of the retrieved articles was performed twice with EndNote (Version X8.0.1, Clarivate Analytics, Philadelphia, PA): first per searched database, and then based on the relevant titles. Studies were assessed based on title, and if irrelevant excluded by 1 investigator (W.D.L.). Afterward, abstracts were screened by 2 independent investigators. Full-text versions of the selected articles were read, and if not relevant excluded independently by 2 investigators. Relevant articles were selected on criteria for studied group and intervention. Patients or animals must have had objectively determined or induced FI. The intervention must have been with some sort of cells, or biocompatible scaffold, with or without the use of trophic factors. Due to the small number of included articles and the variation in reported outcomes, no outcome-related criteria could be used to perform a meta-analysis and the Population, Intervention, Comparison, Outcome and Study design framework for clinical questions could not be followed. Non-English articles were assessed based on available English title

TABLE 1
Comprehensive search strategy, MeSH terms, and words or word combinations searched in title and abstract

Search field	Search terms
MeSH	Fecal incontinence *AND* Biocompatible materials OR regenerative medicine OR bioengineering OR cell- and tissue-based therapy OR biomedical engineering
Title/abstract[a]	Fecal incontinence OR fecal incontinence OR anal dysfunction OR anal sphincter insufficiency OR anal incontinence OR stool incontinence OR anal sphincter damage OR sphincter muscle injury OR anal sphincter *AND* Regenerative medicine OR cell-therapy OR cell therapy OR cell-based therapy OR cell based therapy OR myogenic satellite cell OR myoblast cell OR myoblast OR cell delivery OR muscle progenitor cell OR fibroblast OR stem cell OR muscular cell OR muscle cell OR autograft OR muscle regeneration OR tissue engineering OR TE OR bioengineering OR bio-engineering OR bio engineering OR biological engineering OR tissue regeneration OR biomaterial OR bioactive scaffold OR tissue remodeling OR tissue repair OR tissue construct OR bioengineered construct OR IAS construct OR internal anal sphincter construct OR EAS construct OR external anal sphincter construct OR artificial anal sphincter OR biocompatible material OR tissue therapy OR biomedical engineering OR clinical engineering OR myogenic OR muscle derived OR mesenchymal OR autologous OR allogeneic OR bone marrow-derived

EAS, internal anal sphincter; *IAS*, internal anal sphincter; *TE*, tissue engineering.

[a] All terms were searched in singular and plural spelling as well as alternative spelling.

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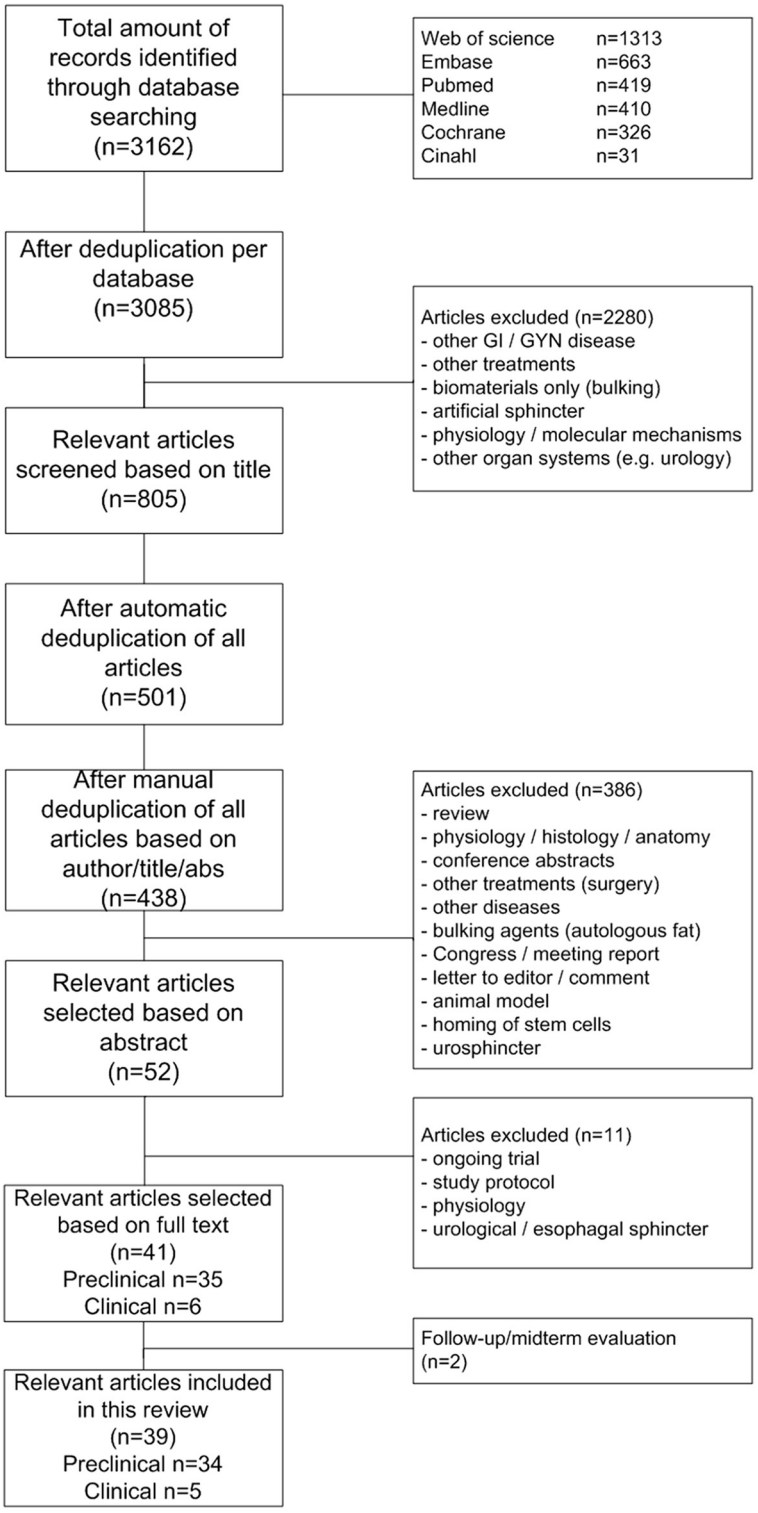
FIGURE 1
Flowchart of search, screening and selection process of studies



Systematic selection of articles and main reasons for exclusion. Figure is based on PRISMA 2009 flow diagram.[59]

GI, gastro-intestinal; *GYN*, gynecological.

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and abstract. If found relevant, the non-English articles were translated by a native speaker to facilitate quality assessment and data extraction.

Assessment of risk of bias

The quality of the articles was assessed using the items of the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias (RoB) tool[14] for the preclinical studies and the Cochrane RoB tool for randomized trials for the clinical studies.[15] It is important to note that while the SYRCLE RoB tool is currently the only tool available to help determine the RoB in animal studies, it is relatively new and not yet validated. All studies were evaluated by 2 of the 3 independent investigators (M.H.K., A.M.R-Z., W.D.L.). In case of disagreement on data selection or quality assessment, consensus was reached involving a third independent investigator followed by discussion. We used the quality assessment to divide the articles into 3 groups: high quality (or low RoB), intermediate quality (or intermediate RoB), and low quality (or high RoB). Each RoB item was scored with a 1 when the answer was "yes" and corresponded to a low RoB, or a 0 when the answer was "no" and corresponded to a high RoB. If a study characteristic was not mentioned in the article, it was scored with 0 or "no." The preclinical studies were assessed using the 10 items of the SYRCLE RoB tool and articles were categorized according to the total score: $\geq$7 was defined as high quality, 5 or 6 as intermediate quality, and $\leq$4 as low quality. Similarly, the clinical studies were assessed with the 7-item Cochrane RoB tool and categorized as high quality when scored $\geq$6, intermediate quality when scored 4 or 5, and low quality when scored $\leq$3.

Results

Study selection

The comprehensive literature search retrieved 3162 articles. Of these articles, 2280 were excluded based on title. A total of 444 hits were excluded after automatic and manual deduplication. The remaining 438 abstracts were assessed and finally 40 full-text articles were included in this systematic review. The

TABLE 2
Overview of quality category, number of subjects, studied group, type of injury, intervention, control, and outcome of all included preclinical studies

Article	Quality category	No. of animals	Animal	Type of injury and objective confirmation	Cell type	Scaffold/factor/ surgical repair	Control	Outcome
Aghaee-Afshar et al,[46] 2009	Intermediate	35	Rabbits	EAS sphincterotomy, clinical examination and EMG	BMSC/hUCM cells	NA	No intervention, medium only and saline	EMG, histology
Bisson et al,[24] 2015	Low	26	Rats	Cryoinjury, not objectively confirmed	Myoblasts	NA	No intervention, saline injection	Histology, anal manometry under electrostimulation
Bohl et al,[32] 2017	Intermediate	20	Rabbits	Hemisphincterectomy, anorectal manometry	Smooth muscle cells from rabbit IAS + enteric neural progenitor cells	Implantation of autologous biosphincter	No intervention, sham surgery without implant	Anorectal manometry (in vivo), histology
Craig et al,[55] 2010	Low	4	Rodents	None, intact EAS	Myoblasts	NA	NA	Histology
Ding et al,[48] 2016	Low	Unclear	Rats	Cut anal sphincter, fecal contamination of furs	BMSC	After end-to-end surgical repair; with ADM + galectin-1 induction of BMSCs	Repair only, repair + ADM	Histology, microscopic observation of growth
Elmi et al,[35] 2014	Intermediate	12	Rabbits	Subtotal external sphincterectomy, resting pressure on EMG	Muscle progenitor cells	NA	Saline injection	Sphincter EMG, manometry, MRI, histology
Fitzwater et al,[33] 2014	Low	40	Rats	Sphincter transection, not objectively confirmed	Myogenic stem cells	After surgical repair	Saline injection	Histology, volumetric analysis, force generation in electrical field
Hashish et al,[56] 2010	Low	10	Mice	None, implantation subcutaneous in back of mice	Smooth muscle cells from mouse IAS	Bioengineered IAS construct + FGF-2	Bioengineered IAS construct + saline	Histology, morphologic assessment
Inoue et al,[44] 2018	Low	18	Rats	Removal of half of IAS and EAS, anal pressure on postoperative day 1	ADSC sheets	NA	No intervention	Anal manometry, histology
Jacobs et al,[20] 2013	Low	33	Rats	Transected anal sphincter of $\geq$5 mm, not objectively confirmed	Myogenic stem cells	After surgical repair; with FGF, EGF, or HGF	Saline injection	Safety assessment: samples of lung/liver, histology
Kajbafzadeh et al,[21] 2010	Intermediate	21	Rabbits	External anal sphincterotomy, not objectively confirmed	Muscle progenitor cells	NA	Saline injection	EMG, manometry, and histology
Kajbafzadeh et al,[47] 2016	Intermediate	8	Rabbits	Excision of entire EAS, change from beaded to semiliquid stool	Myogenic stem cells (satellite cells)	Decellularized matrix of EAS	Decellularized matrix of EAS vs no transplant	EMG, electron microscopy, and histology

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(continued)

TABLE 2
Overview of quality category, number of subjects, studied group, type of injury, intervention, control, and outcome of all included preclinical studies
(continued)

Article	Quality category	No. of animals	Animal	Type of injury and objective confirmation	Cell type	Scaffold/factor/ surgical repair	Control	Outcome
Kang et al,[34] 2008	Low	15	Rats	Cryoinjury, not objectively confirmed	Muscle-derived stem cells	NA	No intervention, no injury nor intervention	In vitro contractility, immunohistochemical staining
Kang et al,[49] 2013	Intermediate	10	Dogs	Partial extraction of 25% of posterior IAS and EAS, not objectively confirmed	Myoblasts	PCL beads	No intervention	CMAP measurement, manometry, histopathology
Kuismanen et al,[43] 2018	Low	60	Rats	Sphincter cut (IAS, EAS, and anal mucosa), not objectively confirmed	Human ADSC	After repair and in Bulkamid (Contura International A/S, Denmark)	Saline injection, Bulkamid alone, human ADSC + saline	Anal manometry, μCT imaging, 3D imaging, histology
Lane et al,[36] 2013	Intermediate	33	Rodents	Proctoepisiotomy, not objectively confirmed	Myogenic stem cells	After layered surgical repair	Layered surgical repair + saline injection	Anal pressure, EMG, tissue analysis
Li et al,[54] 2018	Intermediate	60	Rats	Transection of IAS and EAS + resection of tissue, dietary behavior and defecatory condition	BMSC	EA	Sham surgery + saline injection, injury + EA + saline injection, injury + saline injection	Histology, measurement of SDF-1/MCP-3 for homing of cells
Lorenzi et al,[22] 2008	Intermediate	24	Rats	Sphincterotomy, observation of feeding and defecation behavior	BMSC	After surgical repair	Sham surgery, surgical repair + saline injections	Histology, in vitro contractility
Mazzanti et al,[38] 2016	Low	32	Rats	Sphincterotomy, not objectively confirmed	BMSC	After surgical repair	Repair + saline injection, sham operation + saline injection	In vitro contractility, histology
Miyasaka et al,[28] 2011	Low	16	Mice	None, implantation subcutaneous in back of mice	Smooth muscle cells from mouse IAS	Implantation of IAS rings in back with osmotic FGF-2, VEGF, or PDGF pump	IAS rings without growth factor pump	In vitro physiologic functionality, histology
Montoya et al,[19] 2015	High	80	Rats	Midline anal transection, not objectively confirmed	Myogenic stem cells	PEG hydrogel matrix scaffold	No intervention, injection with scaffold + saline, injection of type 1 collagen	In vitro contractility, muscle volume
Oh et al,[50] 2015	Intermediate	15	Dogs	Resection 25% of posterior IAS and EAS, anal manometry and CMAP	Myoblasts	PCL beads	No intervention, sham surgery: skin incision around sphincter	In vitro contractility, CMAP, histology

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(continued)

TABLE 2
Overview of quality category, number of subjects, studied group, type of injury, intervention, control, and outcome of all included preclinical studies
(continued)

Article	Quality category	No. of animals	Animal	Type of injury and objective confirmation	Cell type	Scaffold/factor/ surgical repair	Control	Outcome
Oh et al,[53] 2015	Intermediate	10	Dogs	Resection 25% of posterior IAS and EAS, anal manometry and CMAP	Myoblasts	bFGF-loaded PCL beads	Injection of autologous myoblasts	Anal manometry, CMAP of pudendal nerve, histology
Pathi et al,[57] 2012	Low	204	Rats	Anal sphincter laceration, not objectively confirmed	BMSC	After repair, local vs IV (tail vein) injection	No injury, injury + local or IV (tail vein) saline injection	In vitro contractility, histology, quantification of cytokines
Raghavan et al,[29] 2010	Low	Unclear	Mice	None, implantation subcutaneous in back of mice	Smooth muscle cells from mouse IAS	Implantation of bioengineered IAS construct in back of mice with FGF-2 pump	NA	In vitro physiologic functionality, histology
Raghavan et al,[30] 2011	Low	Unclear	Mice	None, implantation subcutaneous in back of mice	Smooth muscle cells from human IAS	Implantation of bioengineered constructs from human IAS cells in back of mice with FGF pump	NA	Histology, immunofluorescence, in vitro functionality
Raghavan et al,[31] 2014	Low	Unclear	Rats	Circumferential anal incision and 5 mm dissection of rectum, not objectively confirmed	Smooth muscle cells from human IAS and human enteric neuronal progenitor cells	Implantation of IAS construct in place of anal sphincter with abdominal insertion of PDGF	NA	In vitro contractility, immunohistochemical characterization
Salcedo et al,[40] 2013	Low	70	Rats	Sphincterotomy/pudendal nerve crush, confirmation with dissection microscope	BMSC	IM or IV injection	IM or IV saline injection	Anal manometry, EMG, immunofluorescence analysis
Salcedo et al,[39] 2014	Low	50	Rats	Excision of 25% of IAS and EAS, confirmation with dissection microscope	BMSC	IM or serial IV injections	No injury, no treatment, IM or IV saline injection	Anal manometry, immunofluorescence, histology
Sun et al,[41] 2016	Intermediate	16	Rats	Excision of 50% of ventral anal sphincter, anal manometry	BMSC	After electrical stimulation	No intervention, only daily electrical stimulation for 3 d	Anal manometry, histology
Sun et al,[16,42] 2017	Intermediate	56	Rats	Excision of ventral half of anal sphincter complex, anal manometry	BMSC	Plasmid with gelatin scaffold encoding SDF-1	No intervention, plasmid encoding SDF-1	Anal manometry, histology, immunohistochemistry, morphometry

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(continued)

TABLE 2
Overview of quality category, number of subjects, studied group, type of injury, intervention, control, and outcome of all included preclinical studies
(continued)

Article	Quality category	No. of animals	Animal	Type of injury and objective confirmation	Cell type	Scaffold/factor/ surgical repair	Control	Outcome
Trébol et al,[45] 2018	Low	36	Rats	Animal model of 1 cm extramucosal myotomy, anal manometry and histology	ADSC	After biosuture repair or conventional suture repair	No repair + ADSC	Anal manometry, histology
White et al,[37] 2010	Intermediate	120	Rats	Sphincter transection, not objectively confirmed	Myogenic stem cells	After repair	No repair, repair + saline injection	In vitro contractility
Zhou et al,[18] 2012	Low	30	Rats	Sphincter cut, observation of defecatory condition	BMSC	Repair with ADM	Only repair, repair + ADM	Histology

ADM, acellular dermal matrix; *ADSC*, adipose-derived stem cell; *bFGF*, basic fibroblast growth factor; *BMSC*, bone-marrow mesenchymal stem cell; *CMAP*, compound muscle action potential; *EA*, electroacupuncture; *EAS*, external anal sphincter; *EGF*, endothelial growth factor; *EMG*, electromyography; *FGF*, fibroblast growth factor; *HGF*, hepatocyte growth factor; *hUCM*, human umbilical cord matrix; *IAS*, internal anal sphincter; *IM*, intramuscular; *IV*, intravenous; *MCP*, monocyte chemotactic protein; *MRI*, magnetic resonance imaging; *NA*, not applicable or not mentioned in full-text article; *PCL*, polycaprolactone; *PDGF*, platelet-derived growth factor; *PEG*, polyethylene glycol; *SDF*, stromal cell-derived factor; *VEGF*, vascular endothelial growth factor; *3D*, 3-dimensional; *μCT*, microcomputed tomography.

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2 independent investigators disagreed about the inclusion or exclusion of a total of 77 abstracts. Consensus was reached in all cases involving a third independent investigator. Due to the limited number of articles included in this review, no articles were excluded based on poor quality. The 41 articles that were included in this review contained 34 preclinical studies,[18–22,24,28–50,53–57] 1 preclinical midterm evaluation,[16] 5 clinical studies,[23,25–27,58] and 1 clinical follow-up.[17] The number of excluded articles per step and main reasons for exclusion are depicted in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart (Figure 1). One Chinese article[18] was found relevant based on English title and abstract. The full-text article was assessed on quality, and data were extracted after translation by an independent native speaker.

The articles included contain a broad variety of studied groups, interventions, and outcome measurements (Tables 2 and 3), for this reason we could not perform a meta-analysis.

RoB of included studies

After quality assessment, the articles were divided in 3 groups (Table 4). Following the SYRCLE RoB tool for animal studies, 1 of the 34 preclinical studies was classified as high quality, 14 as intermediate quality, and 19 as low quality. The 2 independent investigators disagreed about the quality assessment in 14 cases (11 preclinical studies, 3 clinical studies). Consensus was reached on the different items of the SYRCLE RoB tool or the Cochrane RoB tool in all cases after involvement of a third independent investigator.

The poor quality of the preclinical studies was because none of the investigators or caregivers were blinded to the intervention, only 1 study randomly assessed the outcome,[19] and in only 3 studies the investigators were blinded to the outcome parameters[20–22] (Figure 2). Most of the animal studies had equal baseline characteristics (n = 29; 85.3%) and were free of selective outcome reporting (n = 29; 85.3%).

The assessed clinical articles were found to be of intermediate or low

quality (Table 3). More than half of the studies did not use randomization or blinding of the intervention. Three out of the 5 included clinical trials adequately addressed incomplete outcome data and were free of selective outcome reporting (Figure 3). The strongest clinical study was the double-blinded, randomized controlled trial from Sarveazad et al,[23] which scored 5 out of 7 points following the Cochrane RoB tool.

Comment

RM for FI

This is the first systematic review providing a complete and critical overview of the available research on RM as a treatment for FI. Due to a wide variation in the studied groups, interventions, and outcome measurements, it was not feasible to perform a meta-analysis. For the same reasons, results from individual articles should be interpreted with care. This review summarizes the results of the articles included, taking into account the quality of the studies, which was assessed using the SYRCLE RoB tool for preclinical studies and the Cochrane RoB tool for clinical studies. The RoB assessment often indicated the poor quality of preclinical and clinical studies, which is not surprising since the field of RM in FI is still in its infancy. The goal of RM as a therapy for FI is to restore the function of the anal sphincter by targeting smooth and skeletal muscle tissues. To better understand the potential of RM treatments for FI, we discuss individual articles according to the type of intervention used: cells, scaffolds or trophic factors (coadministration of trophic factors with cells and/or scaffolds).

Cells

Cells can be used to promote repair and regeneration of tissues either by directly replenishing the tissues and/or by releasing trophic factors to attract and activate other cells to do the job (homing effect). Smooth muscle cells, their precursors (ie, myoblasts, muscle progenitor cells, and muscle-derived or myogenic stem cells), adipose-derived stem cells (ADSC), bone-marrow

TABLE 3
Overview of quality category, number of patients, studied patient group, intervention, control, and outcome of all included clinical studies

Article	Quality category	Patients (objective confirmation of FI)	No. of patients	Cell type	Scaffold/factors/ surgical repair	Control	Outcome
Boyer et al,[27] 2018	Low	Women with severe FI due to sphincter deficiency (endoanal ultrasonography)	24	Autologous myoblasts	NA	Local saline + 1.6% albumin injection	Questionnaires, anal manometry, endosonography, and MRI
Frudinger et al,[25] 2010 Frudinger et al,[17] 2015	Low	Women with refractory FI [third-/fourth-degree tears] due to obstetric trauma (defect on anal endosonography)	10	Autologous skeletal muscle-derived cells/ myoblasts	After electrical stimulation	NA	Symptom diary, Wexner score, QoL questionnaire, endosonography, anorectal physiology Frudinger, 2015: same outcome measurements as Frudinger, 2010, and additionally: manometry at 1, 2, 5 y
Khafagy et al,[58] 2017	Low	Patients with FI due to defective EAS $\leq$half of EAS circumference (EAS defect on endoanal ultrasonography)	40	Bone marrow–aspirated concentrate (mononuclear cells)	After OASR	Case-control: historical group of patients with OASR alone	Wexner score, endoanal sonography
Romaniszyn et al,[26] 2015	Low	Patients with moderate-severe FI due to sphincter insufficiency (reduced basal and squeeze pressure on anorectal manometry)	10	Autologous myoblasts/ myogenic stem cells	With bFGF in culture medium	NA	Anorectal manometry, endorectal EMG, ERUS, questionnaires
Sarveazad et al,[23] 2017	Intermediate	Patients with FI, confirmed with endorectal ultrasonography (Wexner score and endorectal ultrasonography)	18	ADSC	After sphincteroplasty	Sphincteroplasty + local saline injection	Wexner score, endorectal sonography, EMG

ADSC, adipose-derived stem cell; *bFGF*, basic fibroblast growth factor; *EAS*, internal anal sphincter; *EMG*, electromyography; *ERUS*, endorectal ultrasound; *FI*, fecal incontinence; *MRI*, magnetic resonance imaging; *NA*, not applicable or not mentioned in full-text article; *OASR*, overlapping anal sphincter repair; *QoL*, quality of life.

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TABLE 4
Number and percentages of preclinical and clinical studies per quality category

Included studies	Low quality, n (%)	Intermediate quality, n (%)	High quality, n (%)	Total
Preclinical	18 (55)	14 (42)	1 (3)	33
Clinical	3 (60)	2 (40)	0 (0)	5
All	21 (55)	16 (42)	1 (3)	38

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mesenchymal stem cells (BMSC), and human umbilical cord matrix (hUCM) cells have been used in both preclinical and clinical studies to treat FI.

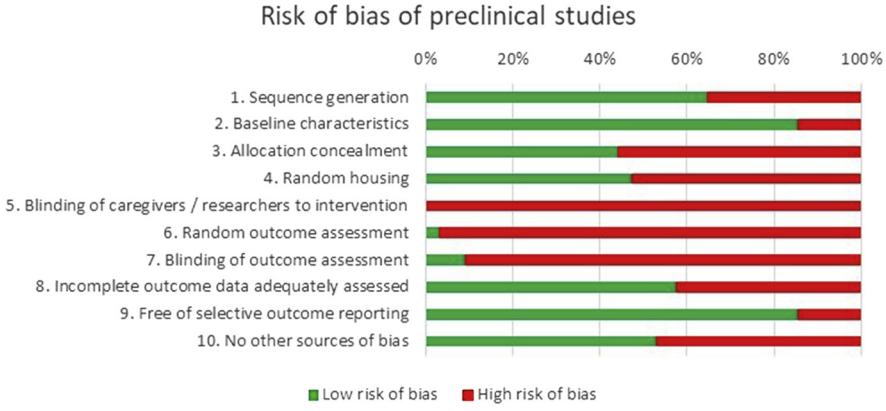
Smooth muscle cells are differentiated and more specialized cells that can be used to repair the damaged anal sphincter. However, they have a limited life span and low ability to replicate; therefore, large quantities of cells would be required causing high donor site morbidity. The precursors of smooth muscle cells are less differentiated and can replicate and differentiate into muscle cells. Their stemness diminishes as the precursor cells are more committed (or differentiated) down into the myogenic lineage. For the purpose of this review, we considered the stemness of the myogenic lineage cells as follows: myogenic stem cells > muscle progenitor cells > myoblasts > muscle cells. The availability of these cells is limited to specific tissues and harvesting them can also cause donor site morbidity. This could be less of a problem when using other sources of adult stem cells such as ADSCs, BMSCs, or hUCM cells. Adult stem cells are undifferentiated multipotent cells that have the ability to self-renew and differentiate to certain lineages. ADSCs are easily available, can be found in high quantities, and can differentiate into muscle cells. BMSCs and hUCM cells are hematopoietic stem cells that can also differentiate into muscle cells but to a lesser extent than myogenic stem cells and ADSCs.

Results from preclinical and clinical studies using cell-based therapies for FI have been very heterogeneous. In general, the use of muscle progenitor cells and myoblasts show promising and relatively durable results in preclinical[24] and even clinical[17,25,26] research. A preclinical study in the cryoinjured anal sphincter of rats showed that injection of myoblasts increased anal pressure for up to 60 days; however, this is a study with a high RoB due to lack of randomization of animals and blinding of investigators.[24] Most of the clinical studies on FI have used autologous myoblasts. A relatively long-term positive effect of 12 months was subjectively assessed using questionnaires, but there was no change in anal pressure.[25,27] However, a trial with 5-year follow-up period showed a significant reduction in Wexner score, frequency and intensity of FI, and increased anal pressure.[17] A small and uncontrolled clinical trial showed that a combination of myoblasts injection with basic fibroblast growth factor (bFGF) decreased the frequency and intensity of the FI.[26] Results should be confirmed in a separate study.

Smooth muscle cells have been used to develop bioengineered IAS constructs. The preclinical studies have used cells from human or animal IAS, cultured them in vitro, and used histological and functional outcome measurements. Four studies tested basal tone in vitro[28–31] and 1 tested basal tone in vivo.[32] The bioengineered IAS restored the basal tone to baseline values in vivo after 3 months of implantation in rabbits.[32] Histological analysis confirmed the presence of smooth muscle cells, vascularization, and innervation in the engineered constructs.

Muscle-derived stem cells, or myogenic stem cells, have been used in preclinical studies with varying results. These cells increased the mean striated muscle volume of repaired EAS in rats, which was equal to the saline control group.[33] Regeneration of myofibers, and eventually smooth and skeletal muscle, has also been reported in rodents.[34,35] However, the effect of these cells on functionality seems to be of limited durability. In 3 preclinical studies, myogenic stem cells increased muscle contractility 2 weeks after

FIGURE 2
Risk of bias graph for all included preclinical studies



Risk of bias (RoB) graph of each RoB item from SYRCLE RoB tool for animal studies that was applied to all included preclinical studies and scored by 2 independent investigators. For each item, "yes" correlates to low RoB and scores 1, while "no" correlates to high RoB and scores 0.

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injection compared to saline or non-injected controls.[21,35,36] Unfortunately, this increase was not maintained after 4 weeks of follow-up. On the other hand, White et al[37] showed a sustained increase of in vitro contractility after 90 days with surgical repair followed by local injection of myogenic stem cells in rats.

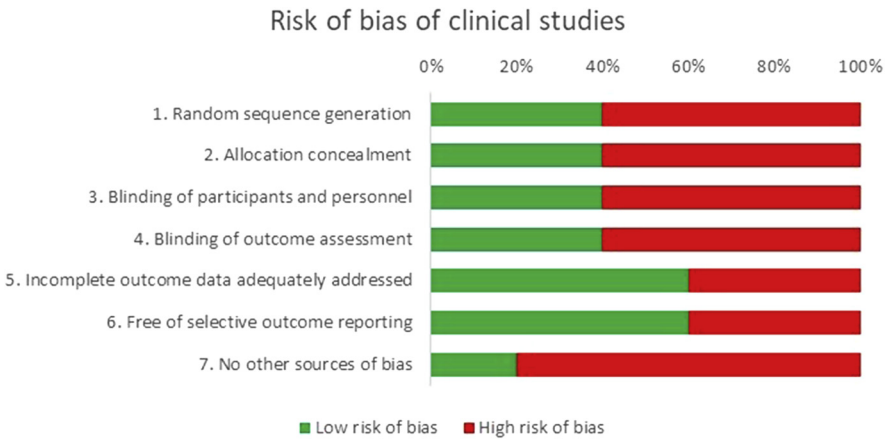
Other types of adult stem cell used preclinically as a treatment for FI are BMSCs. BMSCs are mesenchymal stem cells obtained from bone-marrow tissues. BMSCs have been used in 11 rat studies,[16,18,22,38–42,48,52,54,57] 3 of which compare local versus intravenous injection of BMSCs.[39,40,57] Regardless of mode of injection,[39] injection of BMSCs reduced the amount of fibrotic tissues and increased the percentage of muscle tissues compared to saline or nontreated controls.[16,22,41,42] In these studies, the follow-up time was short, the study quality was intermediate or low. Additionally, no clinical studies have been performed using BMSCs. Therefore, it is still early to say anything about the use of BMSCs as a treatment for FI.

ADSCs are other adult stem cells that have been used in 1 clinical trial[23] and in 3 low-quality preclinical studies using rat models.[43–45] Despite increased contractility in rats, Wexner score did not improve in human beings.

hUCM cells have also been used to treat FI in rabbits.[46] hUCM cells are an easily accessible source of cells obtained from the umbilical cord blood than can be cryopreserved until needed. Unfortunately, the study failed to show differences between the electromyography before injection and after injection. The immunological and practical consequences of the use of these allogenic cells in human beings are not discussed in the study and should be investigated in further research.

In summary, myoblasts have been the most studied cells for FI and show the most promising results in human beings, also over a long follow-up period. A short-term effect of myogenic stem cells, smooth muscle cells, and BMSCs has been shown in animal models but the poor quality of the research means that results need to be confirmed in longer and better-designed studies. Despite the disappointing results in human beings, ADSCs should not be disregarded, considering their easy accessibility and the good functional results in rats.

FIGURE 3
Risk of bias graph for all included clinical studies



Risk of bias (RoB) graph of each RoB item from Cochrane RoB tool for randomized trials that was applied to all included clinical studies and scored by 2 independent investigators. For each item, "yes" correlates to low RoB and scores 1, while "no" correlates to high RoB and scores 0.

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Scaffolds

The right microenvironment is essential for tissue regeneration and development, so choosing the right scaffold material is crucial for the success of a tissue-engineered construct. Scaffolds are materials that can be used to support the growth, proliferation, differentiation, and adhesion of cells. They can be derived from natural or synthetic polymers and can be used alone or in combination with cells and/or trophic factors. Scaffolds have not been used yet to treat FI in human beings, but preclinical studies have shown good functional results in combination with cells. In these tissue-engineered constructs, investigators have used decellularized or acellular dermal matrices with muscle progenitor cells[47] or BMSCs;[18,48] hydrogels with myogenic stem cells,[19] ADSCs,[43] or BMSC;[16,42] and polycaprolactone (PCL) beads with myoblasts.[49,50]

Acellular matrices are natural materials or biological grafts obtained after the decellularization of tissues. These biological grafts can be sterilized and chemically modified according to the clinical application. An example of such biological grafts currently used in the treatment of human rectal prolapse is the porcine decellularized small intestine matrix or Biodesign Rectopexy graft (Cook Medical, Bloomington, IN). Decellularized matrices have the optimal extracellular matrix protein content and architecture to support cellular growth and can promote tissue regeneration.[51] For FI, acellular matrices have been used in 3 rodent studies.[18,47,48] A combination of BMSCs with acellular dermal matrices showed good histological outcomes in 2 rat studies of low quality and with no functional outcomes.[18,48] Another study using decellularized EASs seeded with myogenic stem cells in rabbits showed higher response on electromyography after 2 years compared to controls without cells.[47] Although promising, the benefit of acellular matrices compared to cells alone was not investigated.

Hydrogel scaffolds have also been used in preclinical studies. Hydrogels are highly water-absorbable polymers that can be natural or synthetic, and resorbable or not. Biocompatible hydrogels are being extensively used as cell-delivery scaffolds because they facilitate cell survival and provide a 3-dimensional

framework that can be easily injectable. To treat FI, studies have been performed using hydrogels in combination with cells and/or trophic factors (that will be discussed in the next section). There have been 2 reported studies in rats using synthetic hydrogels: the commercially available Bulkamid or polyacrylamide hydrogel with ADSCs,[43] and a polyethylene glycol (PEG)-based hydrogel with myogenic stem cells.[19] Bulkamid is a viscoelastic nondegradable hydrogel that was designed to provide a bulking effect and is currently used for the treatment of female urinary incontinence. The study showed that injected ADSCs with or without Bulkamid equally improved anal sphincter resting and contraction pressure after 4 weeks of treatment, but the Bulkamid-encapsulated cells showed less inflammatory reaction than cells alone.[43] The use of Bulkamid with ADSCs seems to be a good option to treat FI. However, there is only 1 preclinical study that was of low quality and the long-term effect in the function of the anal sphincter with a nonresorbable material is unknown. Using a resorbable hydrogel with cells might promote tissue regeneration in a better way, as suggested by the study from Montoya et al.[19]

In this study, authors used a PEG-based hydrogel to deliver myogenic stem cells to the injured EAS in rats. This PEG hydrogel is a synthetic material that is biocompatible, biodegradable, and promotes vascularization. This material is liquid at room temperature and forms a gelatin-like structure at body temperature that facilitates the injection and delivery of the construct to the right tissue site. After 12 weeks of treatment, rats receiving this scaffold plus myogenic stem cells showed improved contractile function. Increased volume of striated muscle with continuous muscle fibers in the sphincter were clearly seen in the histological analysis.

Finally, both Kang et al[52] and Oh et al[50] used a local injection of myoblasts with PCL beads in a dog model and showed better sphincter contractility compared to the nontreated dogs after 3 months of treatment. The beads used in these studies were made of PCL, a biodegradable and biocompatible synthetic polymer used extensively in clinics as it is the main component of some sutures, eg, Monocryl (Ethicon, Cornelia, GA). This synthetic material is relatively inexpensive, can be easily processed,[52] and is Food and Drug Administration—approved for certain applications.

Scaffolds combined with cells significantly improve contractility in all preclinical studies for FI. Whether these results can be translated to human beings still needs to be studied in clinical trials. A study design comparing an intervention with the combination of scaffold and cells vs only cells would also be useful to assess the benefit of adding a scaffold.

Trophic factors

Trophic factors, such as growth factors and cytokines, can be used to promote proliferation, differentiation, and homing of cells.

They can also help avoid poor vascularization and tissue inflammation. Until now, growth factors have been used in 5 preclinical studies[28−31,53] and 1 clinical trial.[26] Although the study design was not always adequate, results seem to be better with the coadministration of a growth factor. To promote neovascularization a combination of growth factors can be added to cells and/or scaffolds. Miyasaka et al[28] compared different growth factors—vascular endothelial growth factor, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) —in the implantation of a bioengineered IAS in mice. Results showed higher in vitro contractility in the vascular endothelial growth factor and PDGF groups, with no difference in the degree of neovascularization. In another mouse study, Raghavan et al[29−31] implanted a bioengineered IAS with a growth factor pump with FGF, FGF-2, and PDGF. Constructs implanted in the backs of mice, or perianally, generated basal tone 21−28 days after implantation. These studies lacked a control group. Oh et al[53] compared myoblasts alone to a combination of bFGF-loaded PCL beads and myoblasts in a dog model. Contractile anal pressure was significantly higher in the bFGF-loaded bead group after 3 months. The long-term effects have not been investigated and no comparison was made between bFGF-loaded beads and beads alone in this study. However, to eliminate the possible bias caused by the bulking effect of the beads, previous studies by the same group showed the benefit of using cells compared to PCL beads.[50,52] A small clinical trial added bFGF to the culture medium of myoblasts to facilitate proliferation and inhibit spontaneous myocyte formation before implantation,[26] but no comparison was made with myoblasts without bFGF.

A complicating factor in the treatment of FI is the long period of time between the original injury and the actual treatment. Proinflammatory cytokines are less present in the affected tissue, which causes suboptimal homing of cells. Investigators have used cytokines and electrostimulation to mimic recent injury. In a rat study, local injection of BMSCs, preceded by 3 days of electrical stimulation, increased the anal resting pressure compared to electrical stimulation alone.[41] However, contraction pressure did not increase significantly and there was no control group with BMSCs alone. Local coadministration of cytokines improved the homing of cells in other rat models.[16,42] In these back-to-back studies, the authors showed that a stromal cell-derived factor (SDF)-1 plasmid, with or without BMSCs, can increase anal pressure and promote muscle regeneration as seen histologically with a better organized architecture and less fibrotic tissues than controls. No differences were seen between plasmid combined with cells or scaffold, suggesting that the use of an SDF-1 plasmid might be sufficient to repair an injured anal sphincter. The time between injury and intervention in all 3 studies was 3 weeks, which is comparable to approximately 2 years in human beings and thus a representative time span. In a different approach, Li et al[54] sought to attract cells by combining BMSCs with electroacupuncture in a rat model of FI. They showed that this combination increased the capillary density, and the expression

of SDF-1 and the monocyte chemotactic protein-3, 2 factors that promote cell homing.

Altogether, the treatment of FI with a combination of cells and trophic factors is just beginning. Current studies demonstrate better functional and histological results when adding growth factors, cytokines, or electrostimulation. This indicates a beneficial effect in preclinical studies and calls for more research in this area.

Conclusion

The etiology of FI is multifactorial and conventional treatments are not satisfactory. RM has been increasingly studied as a new treatment option. In this systematic review, the efficacy of RM to treat FI could not be determined due to the high RoB and heterogenicity of the available preclinical and clinical studies. These findings call for more studies with better designs that could actually help the translation of RM to the clinic as an alternative to current treatments for FI. ■

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Appendix A. Complete search strategy PubMed

Search strategy PubMed

Search component 1: Fecal incontinence. "Fecal Incontinence"[Mesh] OR fecal incontinence*[tiab] OR faecal incontinence*[tiab] OR anal dysfunction*[tiab] OR anal sphincter insufficienc*[tiab] OR anal incontinence*[tiab] OR stool incontinence*[tiab] OR anal sphincter damage*[tiab] OR sphincter muscle injur*[tiab] OR anal sphincter injur*[tiab] OR anal sphincter defect*[tiab] OR injured anal sphincter*[tiab] OR anal sphincter*[tiab]

Search component 2: Regenerative medicine. ("Biocompatible Materials"[Mesh]) OR ("Regenerative Medicine"[Mesh]) OR ("Bioengineering"[Mesh]) OR ("Cell- and Tissue-Based Therapy"[Mesh])) OR ("Biomedical Engineering"[Mesh]) OR regenerative medicine*[tiab] OR cell-therap*[tiab] OR cell therap*[tiab] OR cell-based therap*[tiab] OR cell based therap*[tiab] OR myogenic satellite cell*[tiab] OR myoblast cell*[tiab] OR myoblast*[tiab] OR cell delivery[tiab] OR muscle progenitor cell*[tiab] OR fibroblast*[tiab] OR stem cell*[tiab] OR muscular cell*[tiab] OR muscle cell*[tiab] OR autograft*[tiab] OR muscle regeneration[tiab] OR tissue engineering*[tiab] OR TE[tiab] OR bioengineering*[tiab] OR bio-engineering*[tiab] OR bio engineering*[tiab] OR biological engineering*[tiab] OR tissue regeneration*[tiab] OR biomaterial*[tiab] OR bioactive scaffold*[tiab] OR tissue remodeling[tiab] OR tissue repair*[tiab] OR tissue construct*[tiab] OR bioengineered construct*[tiab] OR IAS construct*[tiab] OR internal anal sphincter construct*[tiab] OR EAS construct*[tiab] OR external anal sphincter construct*[tiab] OR artificial anal sphincter[tiab] OR biocompatible material*[tiab] OR regenerative medicine*[tiab] OR cell based therap*[tiab] OR tissue therap*[tiab] OR cell-based therap*[tiab] OR biomedical engineering*[tiab] OR clinical engineering*[tiab] OR myogenic[tiab] OR muscle-derived[tiab] OR mesenchymal[tiab] OR autologous[tiab] OR allogeneic[tiab] OR bone marrow-derived[tiab]

Appendix B. Complete search strategy Ovid databases

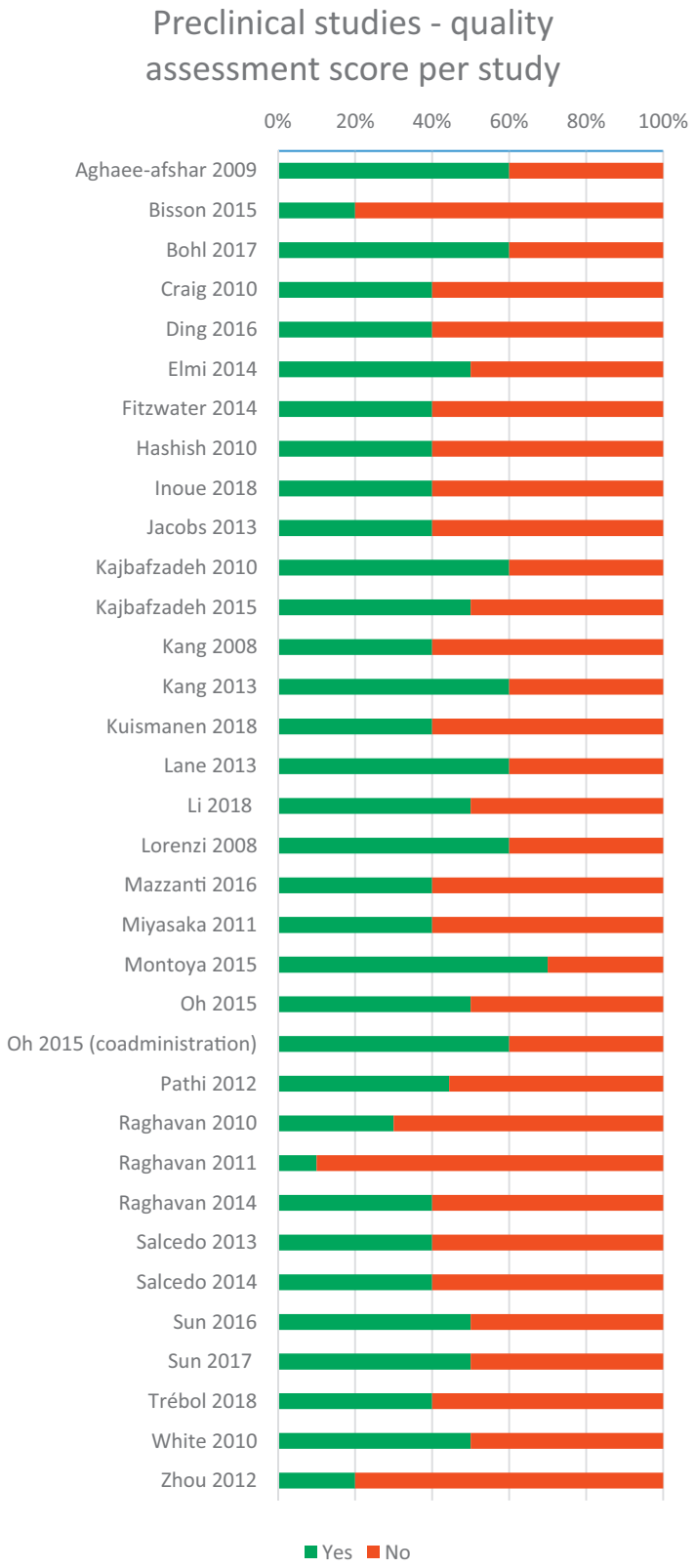
Search strategy Medline/EMBASE:

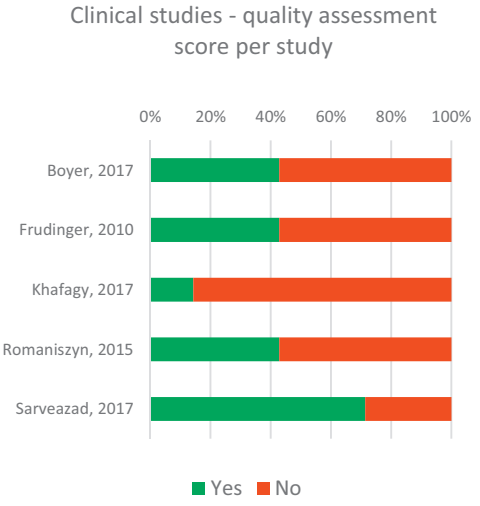
Search component 1: Fecal incontinence. Exp Fecal Incontinence/ OR (fecal incontinence OR faecal incontinence* OR anal sphincter insufficienc* OR anal incontinence* OR stool incontinence* OR anal sphincter damage* OR sphincter muscle injur* OR anal sphincter injur* OR anal sphincter defect* OR injured anal sphincter* OR anal sphincter*).ti,ab.

Search component 2: Regenerative medicine. Exp Biocompatible Materials/ OR Exp Regenerative Medicine/ OR Exp Biomedical Engineering/ OR Exp Bioengineering/ OR Exp Tissue Engineering/ OR Exp "Cell- and Tissue-Based Therapy/ OR regenerative medicine*.ti,ab. OR cell-therap*.ti,ab. OR cell therap*.ti,ab. OR cell-based therap*.ti,ab. OR cell based therap*.ti,ab. OR myogenic satellite cell*.ti,ab. OR myoblast cell*.ti,ab. OR myoblast*.ti,ab. OR cell delivery.ti,ab. OR muscle progenitor cell*.ti,ab. OR fibroblast*.ti,ab. OR stem cell*.ti,ab. OR muscular cell*.ti,ab. OR muscle cell*.ti,ab. OR autograft*.ti,ab. OR muscle regeneration.ti,ab. OR tissue engineering*.ti,ab. OR TE.ti,ab. OR bioengineering*.ti,ab. OR bio-engineering*.ti,ab. OR bio engineering*.ti,ab. OR biological engineering*.ti,ab. OR tissue regeneration*.ti,ab. OR biomaterial*.ti,ab. OR bioactive scaffold*.ti,ab. OR tissue remodelling.ti,ab. OR tissue repair*.ti,ab. OR tissue construct*.ti,ab. OR bioengineered construct*.ti,ab. OR IAS construct*.ti,ab. OR internal anal sphincter construct*.ti,ab. OR EAS construct*.ti,ab. OR external anal sphincter construct*.ti,ab. OR artificial anal sphincter.ti,ab. OR biocompatible material*.ti,ab. OR tissue therap*.ti,ab. OR biomedical engineering*.ti,ab. OR clinical engineering*.ti,ab. OR myogenic.ti,ab. OR muscle-derived.ti,ab. OR mesenchymal.ti,ab. OR autologous.ti,ab. OR allogeneic.ti,ab. OR bone marrow-derived.ti,ab.

APPENDIX C

Figures of the quality assessment per study of all included preclinical and clinical studies





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