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An Affordable Approach of Mesenchymal Stem Cell Therapy in Treating Perianal Fistula Treatment

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Abstract

The application of stem cells to treat perianal fistula due to Crohn's disease has attracted a lot of interest in recent decades. Though still a popular procedure, the existing surgical methods may be an ideal form of therapy since the recurrence rate is high, which affects the quality of life badly. Stem cell therapy offers to be a better solution in treating PF, but the utilisation is often restricted because of the manufacturing cost. Hence in this review, the selection of suitable cell sources, the use of bioreactors and preconditioning MSCs as well as modified stem cells will be discussed for a more affordable as compared with the current MSC therapy towards PF. We anticipate that exploring these approaches may give a complete picture in understanding stem cells in order to make them effective and affordable for long-term therapeutic applications.

Keywords

Manufacturing cost · Refractory Crohn's disease · Stem cells genetic manipulation · Surgery

Abbreviations

ADSCs	Adipose-derived stem cells
AGA	American Gastroenterological Association
bFGF	Basic fibroblast growth factor
BM-MSCs	Bone marrow-derived mesenchymal stem cells
CCL	C–C motif chemokine ligand
CD	Crohn's disease
CD163	Cluster of differentiation 163
CK	Casein kinase
COX2	Cyclooxygenase 2
CPF	Complex perianal fistula
CSF	Colony-stimulating factor
CXCL	Chemokine (C-X-C motif) ligand

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CXCL12	The stromal cell-derived factor-1
CXCR	Chemokine (C-X-C motif) receptor
DC	Dendritic cell
DDL4	Delta-like 4
eASCs	Expanded allogeneic adipose-derived stem cells
EGF	Epidermal growth factor
eIF2	Eukaryotic initiation factor 2
EMT	Epithelial-to-mesenchymal transition
EPG	Epidermal growth factor
EV	Extracellular vesicle
FAS	Fas cell surface death receptor
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptors
FoxP3$^+$ Treg cells	Forkhead box P3$^+$ regulatory T cells
G1 phase	Growth 1 phase
G5k3β	Glycogen synthase kinase 3 beta
GCN2	General control nonderepressible 2
GDF-15	Growth differentiation factor-15
GM	Granulocyte-macrophage
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good Practice Manufacturing
HCAM	Homing cell adhesion molecule
HGF	Hepatocyte growth factor
HLA-G	Human leukocyte antigen G
HUMSCs	Human umbilical cord-derived mesenchymal stem cells
HUVECs	Human umbilical cord vein endothelial cells
IBD	Intestinal bowel disease
ICAM-1	Intercellular adhesion molecule 1
IDO	Indoleamine 2,3-dioxygenase
IEC	Intestinal epithelial cells
IFN-y	Interferon gamma
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor-binding protein
IL	Interleukin
ILT	Immunoglobulin-like transcript
IRF-1	Interferon regulatory factor-1
ITT	Intention to treat
JAK-STAT 1	Janus kinase and signal transducer and activator of transcription 1
K	Keratin
LIFT	Ligation of the intersphincteric fistula tract
M	Matrix protein
MCP-3	Monocyte chemotactic protein 3
miRNAs	MicroRNAs
MMPs	Matrix metalloproteinases
MSCs	Mesenchymal stem cells
NF-κB	Nuclear factor kappa B
NK	Natural killer
Oct4	Octamer-binding transcription factor 4
PAMPs	Pathogen-associated molecular patterns
PDGF	Platelet-derived growth factor
PF	Perianal fistula
PGE2	Prostaglandin E_2
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
S phase	Synthesis phase
SDF-1α	Stromal cell-derived factor-1
SLUG	Snail family transcriptional repressor 2
SNAIL1	Snail family zinc finger 1
Sox2	SRY-box transcription factor 2
TC	Transitional cells
TGF-β	Transforming growth factor beta
Th	T helper
TLR4	Toll-like receptor 4
TNF-α	Tumour necrosis factor alpha
TRAEs	Treatment-related adverse events
Treg cells	Regulatory T cells
TSG6	Tumour necrosis factor-stimulated gene 6
UB-MSCs	Umbilical blood-derived mesenchymal stem cells
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor

VLA-4	Very late antigen 4
VWBR	Vertical-Wheel™ Bioreactors
WJ	Wharton's jelly

1 Introduction

Perianal fistula (PF) is a probable consequence of Crohn's disease (CD) since as many as 26% of patients with CD eventually develop PF within 20 years after the diagnosis. This suggests that the CD cases are perhaps a good tracking parameter for PF incidences (Dudukgian and Abcarian 2011; Schwartz et al. 2019). Traditionally, the occurrence and incidence rate of PF is more common and higher in the Western world such as North America, Europe and Scandinavia as compared to the rest of the world (Ng 2014). However, there have been noticeable changes since the last decade wherein several studies on the epidemiology of CD in the Asia Pacific and developing region revealed an increasing trend, while the rate was stable or rather regressive in the Western countries (Ahuja and Tandon 2010). This shift is likely due to changes in diet, stressful lifestyles and industrialisation in developing countries (Ng 2014).

Current treatment options for PF closely follow its anatomical features. The common one is surgical intervention, namely, fistulotomy, advancement flap procedure and ligation of the intersphincteric fistula tract (LIFT) which aims for a complete healing of fistula although they are less effective in complex cases like transsphincteric fistula (Ji et al. 2021; Limura and Giordana 2015). Despite this, surgeries are complicated with a prolonged recovery period that delays patients from returning to normal life (Sanad et al. 2019). The recovery rate of surgery is less impressive, for example, the success rate for LIFT is only 65% (Lehmann and Graf 2013). Furthermore, it is not uncommon for patients to experience fistula recurrences. Emile et al. (2017) reported that 10.3% of patients relapsed and subjugated themselves to second or third surgeries. Besides healing, there is also a need to address patient satisfaction as well. Side effects like bowel incontinence affect 7% of patients after undergoing surgeries, deteriorating their quality of life by adapting to the adversaries (Dudukgian and Abcarian 2011; Panés and Rimola 2017).

Among the recent revolutionary therapeutic procedures, stem cell therapy showed significant improvement in fistula treatment. Table 1 summarises ten complete clinical trials that were being reported as of 2021, with bone marrow and adipose tissue used as the distributions for the source of mesenchymal stem cells (MSCs). MSCs injected into the tissue surrounding the fistula can restore the damaged tissues primarily through their immunomodulatory effect, stimulating a cascade of immune reactions to foster natural healing (Carvello et al. 2019; Prockop and Oh 2012). The treatment procedure is simpler and takes a shorter time of procedure and hospital stay (Park et al. 2021). Statistically, it boasts a higher success rate, lower recurrences and complete healing with higher patient satisfaction (Ciccocioppo et al. 2019; Herreros et al. 2019).

Despite the superior efficacy of stem cell therapy, its application for PF treatment is underwhelming (Gallo et al. 2020). It suffers from slow industrial growth, yet to be fully realised and made available at a wider scale. High cost of the therapy shrinks its market size, confining stem cell therapy as a last resort for very complex cases only when all other methods fail, thus making it a very niche treatment (Choi et al. 2019). Alofisel, a currently existing medicine specifically for PF based on MSCs derived from allogeneic adipose tissue, costs around $67,000 per dose which some patients need multiple doses for complete fistula closure (Scott 2018). Consequently, the unaffordable cost of treatment is deterring patients from pursuing it, relying on cheaper alternatives. The total cost of treatment is largely dependent on its production cost and numbers of in-process and final release quality assays, as well as on storage (Scott 2018).

A profitable product model is interlinked to the source, isolation and expansion techniques of MSCs. New opportunities like discovering new MSC sources can provide cheaper extraction methods. New advancements in bioreactors previously recruited for bacteria and viruses

Table 1 Clinical studies with stem cell therapy for PF from 2016 to 2021 and their current status

Author (year)/ clinical trial number (if applicable)	Study/clinical trial title	Clinical trial phases	Patient enrolment and injection dosage	Type of cell and its source	Status	Results (if applicable)
Park et al. (2016)	Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohn's disease: a pilot clinical trial	Phase 1	Group 1, n = 3 (10×10^6 cells/mL); group 2, n = 3 (30×10^6 cells/mL)	Allogeneic ADSCs	Completed	At month 8: Complete closure was observed in group 1, 2/3 (67%); group 2, 1/3 (33%)
Panés et al. (2016)/ NCT01541579	Expanded allogeneic adipose-derived mesenchymal stem cell (Cx601) for complex perianal fistulas in Crohn's disease: A phase 3 randomized, double-blind controlled trial	Phase 3	Cx601, n = 107 (120×10^6 cells/mL); placebo, n = 105	Allogeneic ADSCs	Completed	At week 24: 57/107 (53%) Cx601 vs. 43/105 (41%) placebo achieved clinical remission
Dietz et al. (2017)/ NCT01915927	Autologous mesenchymal stem cells, applied in a bioabsorbable matrix, for treatment of perianal fistula in patients with Crohn's disease	Phase 1	12 (20×10^6 cells/mL)	Autologous ADSCs	Completed	At 6 months: 10/12 (83%) achieved complete clinical healing
Panés et al. (2018a, b)/ NCT01541579	Long-term efficacy and safety of stem cell therapy (Cx601) for complex perianal fistulas in patients with Crohn's disease	Phase 3	Cx601, n = 107 (120×10^6 cells/mL); placebo, n = 105	Allogeneic ADSCs	Completed	At week 52: 61/103 (59%) Cx601 vs. 42/101 (42%) placebo achieved clinical remission in modified ITT group
Wainstein et al. (2018)	Stem cell therapy in refractory perianal Crohn's disease: Long-term follow-up	Observational pilot study	9 (100×10^6–120×10^6 cells/mL)	Autologous ADSCs	Completed	At median follow-up of 31 months: 8/9 (89%) patients achieved complete healing
Serrero et al. (2017)/ NCT02520843	Long-term safety and efficacy of local microinjection combining autologous microfat and adipose-derived stromal vascular fraction for the treatment of refractory perianal fistula in Crohn's disease	Phase 1	10 (10.9×10^6–47.8×10^6 cells/mL)	Autologous ADSCs	Completed	At week 48: 80% of patients had clinical responses; 60% of patients had combined remission

Laureti et al. (2020)/ NCT03555773	Refractory complex Crohn's perianal fistulas: A role for autologous microfragmented adipose tissue injection	Prospective pilot study	15 (20 mL microfragmented ADSCs)	Autologous ADSCs	Completed	At 24 weeks: 10/15 (67%) patients achieved combined remission that includes clinical and radiographic assessment; 4/15 (27%) patients had improved condition
Dige et al. (2019)/ NCT03803917	Efficacy of injection of freshly collected autologous adipose tissue into perianal fistula in patients with Crohn's disease	Phase 1	21 (18 mL–104 mL)	Autologous ADSCs	Completed	At 6 months: 12/21 (57%) patients had complete fistula healing; 3/21 (14%) patients had ceased fistula secretion; 1/21 (5%) patients had reduced fistula secretion
Zhou et al. (2020)/ ChiCTR1800014599	Autologous adipose-derived stem cells for the treatment of Crohn's fistula-in-ano: An open label, controlled trial	Phase 2	ADSCs, n = 11 (5×10^6 cells/mL); placebo, n = 11	Autologous ADSCs	Completed	At 12 months: Healing rates were observed in 7/11 (64%) patients in the ADSCs group vs. 6/11 (55%) patients in the placebo group
Barnhoorn et al. (2020)/ NCT01144962	Long-term evaluation of allogeneic bone marrow-derived mesenchymal stromal cell therapy for Crohn's disease perianal fistulas	Phase 1	Cohort 1, n = 5 (10×10^6 cells/mL); cohort 2, n = 5 (30×10^6 cells/mL); cohort 3, n = 5 (90×10^6 cells/mL); placebo, n = 6	Allogeneic BM-MSCs	Completed	At 4 years: Cohort 1, 3/4 (75%); cohort 2, 4/4 (100%); cohort 3, 1/5 (20%); achieved complete clinical fistula closure vs. placebo, 0/3 (0%)
2020/NCT04519671	A phase IB/IIA study of adult allogeneic bone marrow derived mesenchymal stem cells for the treatment of perianal Fistulizing Crohn's disease	Phases 1 and 2	40 (75×10^6 cells/mL)	Allogeneic BM-MSCs	Recruiting	–
2021/NCT04791878	A phase I study of adult allogeneic bone marrow derived mesenchymal stem cells for pediatric perianal Fistulizing Crohn's disease	Phase 1	10 (75×10^6 cells/mL)	Allogeneic BM-MSCs	Recruiting	–

(continued)

Table 1 (continued)

Author (year)/ clinical trial number (if applicable)	Study/clinical trial title	Clinical trial phases	Patient enrolment and injection dosage	Type of cell and its source	Status	Results (if applicable)
2021/NCT04939337	Study to assess the safety and efficacy of allogeneic umbilical cord-derived mesenchymal stem cells (TH-SC01), for treatment of complex perianal fistulas in perianal Crohn's disease	Phase 1	24 (120×10^6 cells/mL)	Allogeneic UC-MSCs	Enrolling by invitation	–
2021/NCT05039411	A phase I study of the safety of allogeneic human umbilical cord mesenchymal stem cells (UC-MSCs) for perianal fistulas in patients with Crohn's disease	Phase 1	7 ($125–150 \times 10^6$ cells/mL)	Allogeneic UC-MSCs	Not yet recruiting	–

Numbers of clinical trials have been conducted to identify the efficiency and the efficacy of MSCs towards PF based on the injection dosage and type of cell source. There are still several clinical trials up until now to prove the safety of MSCs against PF with Crohn's disease

expansion can be modified to sustain a biological environment for the various techniques that exist for cell culture production, with limitations for MSCs (McKee and Chaudhry 2017; Damasceno et al. 2020). Hence, in this review, the market demand and the details of finding a practical and cost-effective approach to transfer MSCs from various sources for treating PF using shorter time are explored.

2 Pathophysiology of Perianal Fistula

A fistula represents a tunnel under the skin which connects two epithelial surfaces. The most prevalent among the fistulas is PF, which typically connects the rectum and drains out to the skin around the anus. Apart from CD, PF also occurs due to infection (Scharl et al. 2016). Multifactorial changes in physical behaviours and biological functions in the rectum area cause a surge in inflammation which induces an inflammatory response, such as activation of macrophages, monocytes and neutrophils (Chen et al. 2018). This leads to the secretion of pro-inflammatory cytokines, chemotactic and cell-activating peptides as well as tissue-degrading enzymes and reactive oxygen radicals, which induce local tissue injury (Scharl et al. 2016).

The release of the pro-inflammatory cytokine, tumour necrosis factor (TNF), stimulates the expression of transforming growth factor-beta (TGF-β) which leads to the production of β-integrin that act as a catalyser to the onset of epithelial-to-mesenchymal transition (EMT) (Scharl and Rogler 2014). EMT redifferentiates epithelial cells located on the inner lining of the rectum into fibroblastic-like cells with migratory capability and penetrates adjacent tissue (Panés and Rimola 2017; Scharl and Rogler 2014). TGF-β also triggers interleukin-13 (IL-13) and increases the secretion of matrix metalloproteinases (MMPs) which are associated with cell-invasive aid (Scharl and Rogler 2014). The overactivation of β-integrin and MMPs marks the birth of the fistula. Once the wastes from the body pile up, during defecation, the intraluminal pressure drives the wastes into subcutaneous tissues (Bataille et al. 2004). The accumulation of wastes leads to the formation of abscesses which are potentially the source of bacterial infection. The luminal pressure makes the tunnel become longer until an external opening is formed (de Zoeten et al. 2013). As a result of this mechanism, the deep penetrating tract develops into PF. Based on the pathophysiology and the severity of fistulas, precise classification of PF in patients has been highlighted in order to come out with the best clinical strategy (Marzo et al. 2015). There are three classifications of PF, which are Parks classification, St James University Hospital classification and American Gastroenterological Association (AGA) classification shown in Fig. 1 (Panés and Rimola 2017).

3 Mechanism of Action of MSCs

The main mechanism of action of MSC entirely is attributed to its paracrine factors such as cytokines, chemokines and growth factors (Park et al. 2018). MSCs release secretomes that help to suppress inflammation, increase cell proliferation and repair damaged tissue (Park et al. 2018). Here we briefly explain the mechanism that is likely to happen upon the injection of MSCs to the side of the fistula. The mechanism of action is also shown in Fig. 2.

3.1 Homing Ability

After injection into the fistula tissue, MSCs enter the vascular system and migrate into the injured site through a homing mechanism (Li et al. 2019; Ullah et al. 2019). Several cell signalling molecules play a crucial role for efficient homing (Spees et al. 2016). For example, the presence of pro-inflammatory cytokines like TNF-α activates endothelial cells in blood vessels to induce intercellular adhesion molecule 1 (ICAM-1), vascular

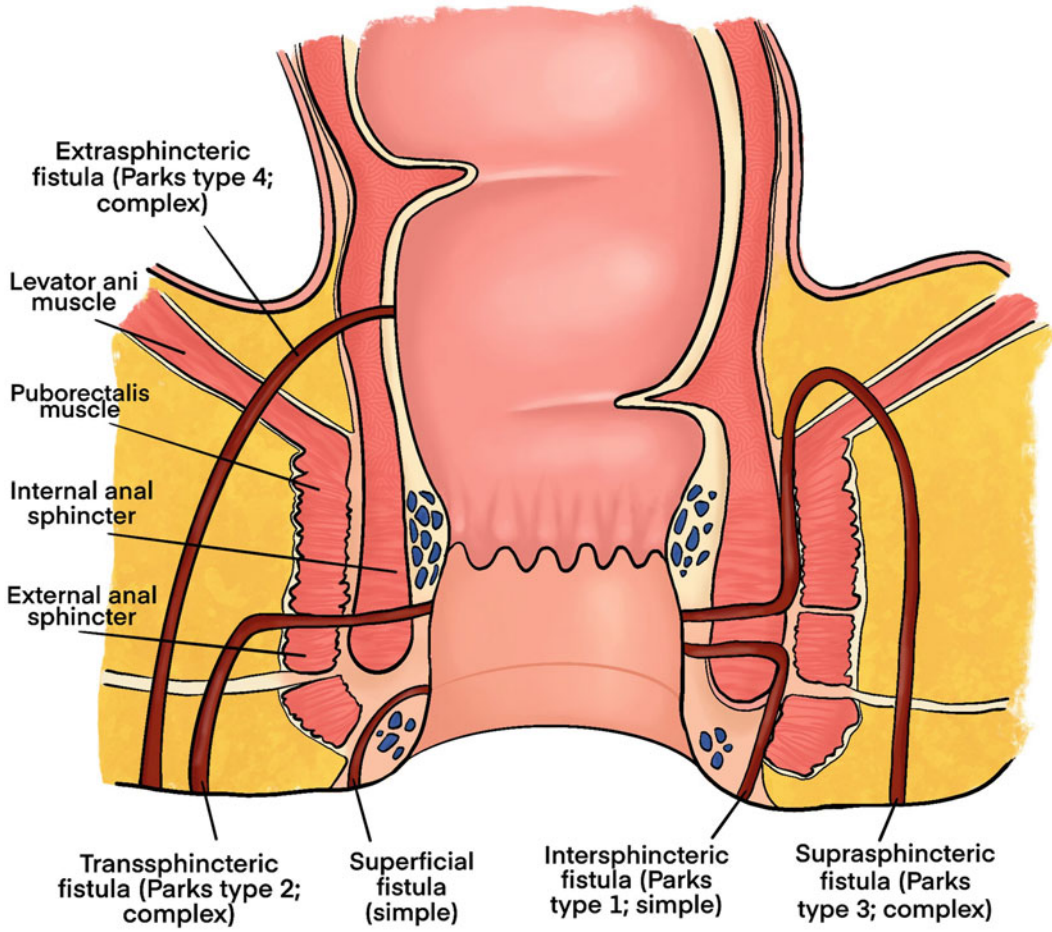

Fig. 1 Classification of PF. Superficial fistula: A fistula without interception with sphincter or muscular structure (Agha et al. 2013). Intersphincteric fistula: Located in intersphincteric plane. Fistula located in between internal anal sphincter until the external anal sphincter (Agha et al. 2013). Transsphincteric fistula: A fistula that will travel through the external sphincter (high or low) to pass some distance distally in the intersphincteric plane before going through the external sphincter (Agha et al. 2013). Suprasphincteric fistula: Fistula that perforates through the intersphincteric plane before piercing the levator ani and descending through the ischioanal fossa (Agha et al. 2013). Extrasphincteric fistula: Fistula starts from the external anal sphincter that tracks across the levator ani to the perineum (Agha et al. 2013)

cell adhesion molecule 1 (VCAM-1) and P-selectin activation for increasing adhesion of MSCs to the endothelial cells (Teo et al. 2012). The movement of MSCs on vascular cell surfaces prompts the upregulation of ligands such as cluster of differentiation 44 (CD44), homing cell adhesion molecule (HCAM) and CD49d (Andreas et al. 2014). Integrins like very late antigen 4 (VLA-4) are formed by galectin-1 to regulate the adhesion of MSCs. Platelet- and neutrophil-derived growth factors such as fibroblast growth factor (FGF) as listed in Table 2 are released by MSCs to interact with basic fibroblast growth factor (bFGF) in endothelial cells to regulate the adhesiveness of galectin-1 to P-selectin (Langer et al. 2009).

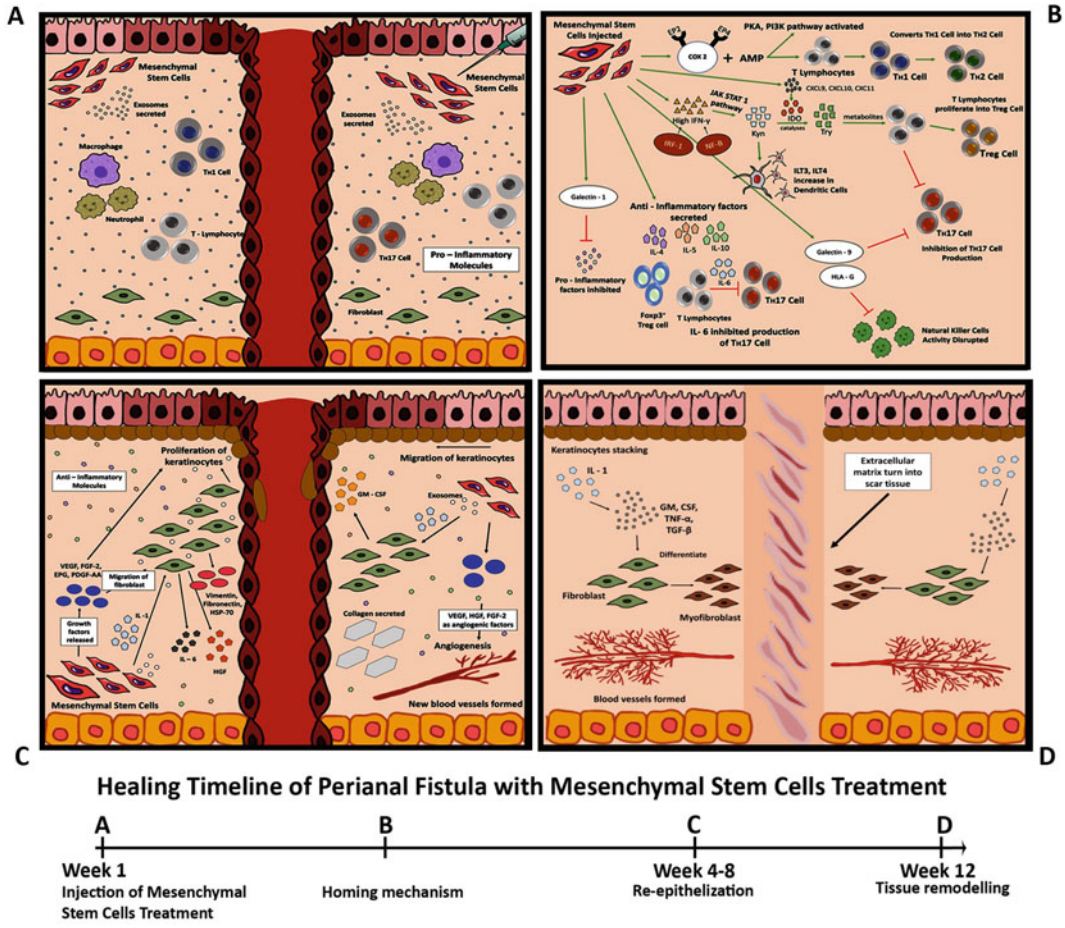

Fig. 2 Healing time of PF with MSCs treatment. (**a**) MSCs were injected into the active inflammation site. The active inflammation site contains an abnormal amount of pro-inflammatory molecules such as TNF-α, IL-1β, TGF-β and others including TH1 and TH17. (**b**) Mechanism of action of MSCs to the active inflammation site including suppressing the production of pro-inflammatory factors and anti-inflammatory factors which are secreted. The production of TH17 is inhibited by the anti-inflammatory factors and Th1 is converted into Th2. Pathway protein kinase (PKA), phosphoinositide 3-kinase (PI3K) and JAK STAT1 activated. (**c**) Re-epithelisation of tissue around the fistula. The abundance of anti-inflammatory molecules balances out the pro-inflammatory molecules. Growth factors such as VEGF, FGF-2, EPG and PDGF-AA are released to assist the migration of fibroblast and the proliferation of keratinocytes to the damaged tissue. The growth factors also contributed to the angiogenesis process, allowing the secretion of collagen. (**d**) Tissue remodelling leaving the extracellular matrix turns into scar tissue and the fistula is healed. New blood vessels completely formed

3.2 Anti-Inflammatory Mechanism

Once it has migrated to the injured site, microenvironment of the injured tissues which consist of pro-inflammatory cytokines secreted during inflammation such as interferon gamma (IFN-y), TNF, IL-1 and IL-17 activates the immunomodulation role of MSCs (Waterman et al. 2010; Sangiorgi and Panepucci 2016). Several immunoregulatory factors like IL-10, prostaglandin E_2 (PGE2), human leukocyte antigen G (HLA-G), indoleamine 2,3-deoxygenate (IDO) 1 and IDO2 and chemokines like chemokine C-X-C motif receptor (CXCL) 9, CXCL10 and CXCL11 will be secreted by MSCs as listed in Table 3 (Li et al. 2018). Once the MSC receptors bind to the

Table 2 Growth factors secreted by MSCs

Author (year)	Growth factors secreted by MSCs	Function
Joel et al. (2019)	HGF	Exert anti-inflammatory signals by causing MSCs to inhibit the proliferation and/or activities of $CD4^+$ Th1, Th17, $CD8^+$ T cell and NK cells
Wang et al. (2014)	bFGF	Proliferates and promotes differentiation of fibroblasts
Delafontaine et al. (2004)	IGF-1	Tissue growth factor with effects to influence blood glucose level
Zhao et al. (2013)	PDGF	Promotes migration of fibroblasts
Tamama et al. (2010)	EGF	Promotes cell regeneration by stimulating cell proliferation
Panek-Jeziorna and Mulak (2020)	FGF-19	Growth factor with potential anti-inflammatory properties
Langer et al. (2009)	FGFR	Interact with bFGF in endothelial cells to arbitrate the adhesiveness of galectin-1 to P-selectin
Zhao et al. (2013)	FGF-2	Attracts leukocyte recruitment to the inflammation site
		Increases the migration of fibroblasts and the functional roles of keratinocytes. Initiates vascularization in the damaged tissue
Ho et al. (2012)	GDF-15	Antiapoptotic, antihypertrophic and anti-inflammatory properties in response to oxidative stress or pro-inflammatory signalling molecules
Zhao et al. (2013)	VEGF	Angiogenesis factor which involves immunology
		Increases the migration of fibroblasts and the functional roles of keratinocytes

The growth factors secreted by MSCs and its function are described. Most of the growth factors show immunomodulatory and anti-inflammatory properties which may benefit in PF treatment

cytokines, chemokines and growth factors in the microenvironment, production of the anti-inflammatory paracrine factors is initiated (Waterman et al. 2010). The paracrine factors will tune the function of the T lymphocytes, macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DC) and B lymphocytes for immunosuppression activities (Wang et al. 2014).

IDO production is activated by the Janus kinase and signal transducer and activator of transcription 1 (JAK-STAT 1) signalling pathway (Ji et al. 2017). In this pathway, the nuclear factor-beta (NF-β) and interferon regulatory factor-1 (IRF-1) bind to upstream IFN-γ-responsive elements of the IDO gene, thus promoting gene expression (Sohni and Verfaillie 2013). IDO behaves as a switch that initiates a cascade of reactions to promote immunosuppression (Luz-Crawford et al. 2013). It directs the monocyte to differentiate into anti-inflammatory and immunosuppressive type 2 macrophage (Francois et al. 2010). Moreover, CXCL12 and CXCR4 secreted by MSCs draw near the T cells to enable IDO to catabolise the tryptophan in T cells (Sohni and Verfaillie 2013). Tryptophan, which is a necessary molecule for T-cell survival, is then broken down into metabolites like kynurenine, quinolinic acid and picolinic acids (Weber et al. 2006). The deficit in tryptophan numbers retards the T-cell multiplication and growth, thus stunting their numbers (Moffett and Namboodiri 2003). This forces a change in the metabolic pathway of ATP production from glycolysis to oxidative phosphorylation and activates a stress response in the immune cells eukaryotic initiation factor 2 (eIF2) and general control nonderepressible 2 (GCN2) through the accumulation of uncharged tRNA (Zhu et al. 2011). Consequently, the arrested cell growth declines their physiological roles, mediating fas cell surface death receptor (FAS)-regulated lymphocyte apoptosis. On the contrary,

Table 3 Paracrine factors secreted by MSCs in PF treatment

Author (year)	Anti-inflammatory factors secreted by MSCs	Function
Schraufstatter et al. (2015)	Complement component C5	Anti-inflammatory cytokine, increases survival of MSCs under oxidative stress
Weiss and Dahlke (2019)	COX2	Anti-inflammatory cytokine, involves in reducing pain
Solodeev et al. (2018)	Fas ligand	Improves cell survival during inflammation tissue damage
Hartung (1998)	G-CSF	Reduces inflammatory activity by inhibiting the secretion or function of the main inflammatory mediators
Luz-Crawford et al. (2013)	IDO 1 & 2	Catabolic enzyme with immunosuppressive properties through kynurenine pathway
Miguel-Hidalgo et al. (2007)	ICAM-1/CD54	Modifies the immunosuppressive functions of MSCs by facilitating the interaction between pro-inflammatory macrophages and MSCs
Lee et al. (2018)	IL-1	Anti-inflammatory cytokine that can inhibit Th17 polarisation
Amorin et al. (2014)	IL-4	Treg cell differentiation and Th2 cells
Pripp and Stanišić (2014)	IL-5	Anti-inflammatory cytokine, increases the development of new nerve cells in the hippocampus and lowered the quantity of potentially damaging inflammation in the brain
Amorin et al. (2014)	IL-10	The most potent anti-inflammatory cytokine, high levels are predicted to involve to the ageing secretome Treg cell differentiation and Th2 cells
Nilsson et al. (2019)	IL-13	Anti-inflammatory cytokine, involves in reversing ageing
Liu et al. (2018)	MCP-3 (CCL7)	Participates in anti-inflammatory responses through binding to its receptors to facilitate the recruitment of immune cells
Park et al. (2006)	PGE2	Vasodilator, reduces inflammation
Luz-Crawford et al. (2013); Mallis et al. (2018)	HLA-G	Inhibits T-cell differentiation into Th1 and Th17. Allows apoptosis of T and B cells
		Stops cytolysis of $CD8^+$ cells and induces development of $CD4^+$, $CD25^+$, $FoxP3^+$ Treg cells
		Disrupts activities of NK cells
Sohni and Verfaillie (2013)	CXCL9, CXCL10, CXCL11, CXCL12	Immunomodulatory roles in MSCs wound healing
Sohni and Verfaillie (2013)	CXCL12, CXCR4	Draws near T cell towards MSCs
Akiyama et al. (2012)	TGF- β	Development and maturation of Treg cell differentiation
Wang et al. (2014)	TSG6	Anti-inflammatory properties
Gieseke et al. (2010)	Galectin-1	Downregulates the pro-inflammatory cytokines TNF-α, IFN-y, IL-2, IL-10
Pripp and Stanišić (2014)	Galectin-9	Anti-inflammatory cytokine, increases the development of new nerve cells in the hippocampus and lowered the quantity of potentially damaging inflammation in the brain
Whelan et al. (2020)	CCL2	Regulates macrophage recruitment, accelerates wound healing

The paracrine factors secreted by MSCs that may involve in PF treatment are stated and its role in PF treatment is also described based on several studies

kynurenine upregulates inhibitory receptors like immunoglobulin-like transcript (ILT) 3 and ILT4 in the DC, while co-stimulating cytokines are suppressed in parallel. HLA-G expressed by MSCs also induce T and B immune cells to undergo apoptosis (Mallis et al. 2018). They further end the cytolysis of antigen-activated $CD8^+$ cells and encourage the development of $CD4^+$, $CD25^+$ and forkhead box $P3^+$ regulatory T cells ($FoxP3^+$ Treg cells).

Galectin-1 secretion by MSCs downregulates the pro-inflammatory cytokines such as IFN-y, IL-2, TNF-α, IL-10 and so on (Gieseke et al. 2010). Together with galectin-9, biological activities of T cells are controlled to reduce T and B lymphocyte numbers (Mallis et al. 2018). Activated MSCs also regulate immunoglobulin (lg) E and IgG concentration by retarding the growth of B cells (Wang et al. 2014). MSCs downregulate IL-2 and IL-15 to keep the natural killer cells dormant, while $CD14^+$ is inhibited by MSCs to stop DC differentiation (Spaggiari and Moretta 2013).

3.3 Cell Proliferation Stage

Activation of GCN2 plays a role in promoting differentiation of Treg cells and downregulates IL-6. Without adequate IL-6, T helper (Th) 17 cells' activities are suppressed (Liu et al. 2020). The tryptophan metabolites, kynurenine, induce the production of tolerogenic DC (Regmi et al. 2019). All the metabolites overall exhibit toxic effects towards $CD4^+$ Th1 and $CD8^+$ T cells but are substantially harmless towards immunosuppressing cells like Th2. This impact prompts T helper cells to start developing into Th2 cells and decrease the development into Th1 cells (Weiss and Dahlke 2019). On the other hand, MSCs block TNF-α secretion by promoting IL-10, and IL-4 secretion also helps in increasing the Treg cell differentiation and Th2 cells (Amorin et al. 2014).

IDO, HLA-G, galectins and other secretomes carry out an extra role of inhibiting the differentiation and development of T cells into Th1 and Th17 (Luz-Crawford et al. 2013). This inhibition forces the macrophages to express growth factors, TGF-β which plays a vital part in the development and maturation of Treg cell differentiation (Akiyama et al. 2012). At the same time, HLA-G also disrupts the protoplasmic activities of NK cells (Mallis et al. 2018).

Furthermore, MSC exosomes can polarise macrophages from pro-inflammatory matrix protein 1 (M1) into anti-inflammatory M2 phenotypes once triggered by the availability of pro-inflammatory cytokines such as chemokines, IL-1β, IL-12 and TNF-α (Murray 2017). It is found that miR-223 in exosomes abates inflammation and helps to speed up healing through macrophage M2 polarisation, while M2 macrophage is brought about by Th2 cytokines and chemokines like TGF-β, IL-10 and M2 markers such as IL-1ra, CD163 and C-C motif chemokine 22 (Murray 2017; Zhuang et al. 2012).

3.4 Re-epithelisation and Tissue Remodelling

MSCs help to remodel the damaged tissues by accelerating wound healing (Nie et al. 2011; Whelan et al. 2020). MSCs will differentiate to fibroblasts and express vimentin, fibronectin and heat shock protein 47 once embedded into the injured site IL-1 secreted by MSCs influences gene expression for other chemokines involved in the metabolic cascade chain like granulocyte-macrophage (GM), colony-stimulating factor (CSF) and TNF-α (Hamilton 2008; Shingyochi et al. 2015). MSCs also differentiate into keratinocytes, producing keratin (K) 5 and K14, integrins, cytokeratin 5, cytokeratin 14, cytokeratin 19, desmoglein 3 and cytokeratin 6α proteins for keratinocyte assembly (Shingyochi et al. 2015). Other than that, they can also differentiate endothelial cells into blood vessel walls (Ebrahimian et al. 2009). Growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EPG), fibroblast growth factor 2 (FGF-2) and PDGF-AA that are secreted by MSCs increase the migration of fibroblasts and the functional roles of keratinocytes (Zhao et al. 2013). VEGF, HGF and FGF-2 also act as angiogenic factors by initiating vascularisation in the damaged tissue.

New blood vessels help in transporting nutrients and oxygen for the cell proliferation of fibroblasts and keratinocytes. At the same time, MSCs secrete IL-1 which directs the migration of fibroblast to the wound, excreting IL-6, HGF and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Zhao et al. 2013).

Lastly, during remodelling, keratinocytes stack up in alignment through epidermal

stratification (Santoro and Gaudino 2005). The mechanical tension from the tissue activates TGF-β, and splice variant fibronectin triggers proto-fibroblast differentiation into smoother myofibroblasts, increasing proliferation. When the wound gap closes, the excess capillaries slowly disappear, leaving behind a completely remodelled tissue (Sorg et al. 2017; Hinz et al. 2001).

4 A more Affordable MSC Therapy in Treating PF

Stem cell therapy was widely investigated in various kinds of diseases, including PF. As compared to the other treatments, stem cell therapy has a relatively high healing rate, which is up to 59% with a low complication rate, 13%–18.5% (Fig. 3). Hence, stem cell therapy is a good alternative for treatment that gives a long-term outcome with fewer relapses (Georgiev-Hristov et al. 2018). Table 4 shows the current conventional treatments and its respective cost. Unfortunately, the high cost appears to be a major bottleneck, causing the limited industrial growth towards a wider market base, especially in the low-income countries. The entire process from the manufacturing process until the delivery of the services requires a high amount of cost and resources, thus causing MSCs as a premium therapy that only PF patients with a higher tier of income could be able to afford. Therefore, several techniques and alternative approaches are being suggested in the following section, making MSCs a more affordable therapy.

4.1 Cell Source

MSCs can be derived from a variety of sources such as the adipose tissue, bone marrow and umbilical cord. Among these various sources, BM-MSCs and adipose tissue-derived MSCs (ADSCs) are commonly utilised in PF treatment due to their easy accessibility (Musiał-Wysocka et al. 2019). Two approaches are involved in obtaining adult MSCs, which are either autologous or allogeneic. Autologous MSCs have the advantage in preventing transplant rejection and performing better cell tolerability; however it is not readily available where it needs to be further isolated and expanded, which may not be suitable in emergency treatment (Molendijk et al. 2015). This leads to allogeneic MSCs which could be pre-cultured in advance and are ready to use anytime. Nonetheless, a healthy donor can be selected to obtain functional and normal MSCs, and these cells may be cryopreserved and readily available for future use (Molendijk et al. 2015).

Cheng et al. (2020) reported that a majority of the reported clinical trials used BM-MSCs and ADSCs in treating PF, with an authorised product available using the latter source. Although these well-studied findings reported a visible healing rate towards PF, several challenges remain unsolved. Firstly, both BM-MSCs and ADSCs recorded low proliferation rates, which were proven by Amable et al. (2014). Besides, age becomes a limiting factor in obtaining high-quality adult tissue-derived MSCs. According to Bustos et al. (2014), using aged BM-MSCs reported a decrease in immunomodulatory activity which was caused by the lowered expression level of chemokine and cytokine receptors that are involved in cell migration. Further, additional steps are needed to extract MSCs from adipose tissue and bone marrow, for example, by bone aspiration and liposuction, and these may increase the risk of contamination if mishandling of samples occurs throughout the workflow (Mazini et al. 2021). With the limitation of adult tissue-derived MSCs, the umbilical cord seems to be an ideal cell source selection in PF treatment. UC-MSCs proved to have a better proliferation rate and cellular migration ability while possessing similar immunomodulatory characteristics with adult tissue-derived MSCs (Omar et al. 2014). For example, WJ-MSCs are reported to have higher expression levels of IL-10, TGF-β and VEGF compared with adult tissue MSCs, which perform better immunosuppressive ability against diseases, and it has been proven since the last decade (Weiss et al. 2008).

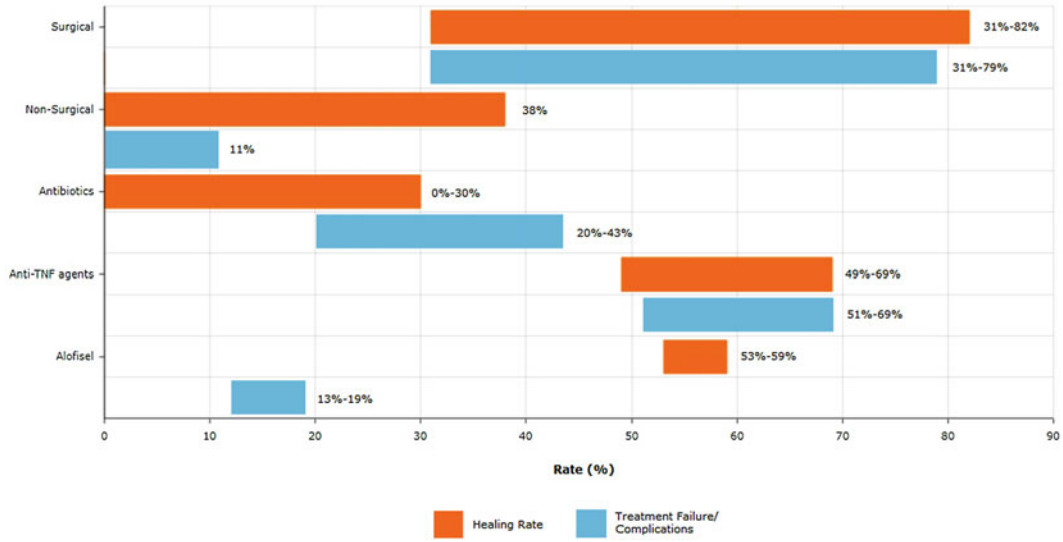

Fig. 3 Graph of treatment success and complication rate of current treatment of PF. Stem cell therapy (Alofisel) has a relatively high healing rate, which is up to 59% with a low complication rate, 13%–19%. Treatment using anti-TNF agents has both high healing and complication rate, which are up to 69%. The healing rate of treatment using antibiotics is low, 0%–30% with high complication rate, which is up to 43%. The healing rate of non-surgical treatment is up to 38% with low complication rate, 0%–11%, while surgical treatment gives highest healing rate, 31%–82% with the highest complication rate, which is up to 79%

4.2 Manufacturing Scales

To produce MSCs in a large quantity as a commercial product for treating PF, a Good Manufacturing Practice (GMP)-compliant production process using a bioreactor is crucial. Traditionally, MSCs are cultured in monolayer flasks, have a simple handling process and are generally low in cost (Rodrigues et al. 2011). However, monolayer culture technology has a higher risk of contamination due to the open system coupled with low cell yield (Mizukami and Swiech 2018). This has prompted for the use of bioreactor for cell expansion to upsize the scale of cell manufacture. Since MSCs are anchorage-dependent, the microcarriers that are present in the bioreactor are used for cell attachment and cell growth by providing higher surface area to volume, allowing a higher yield of MSCs in a shorter time, thus reducing the cost of production (Panchalingam et al. 2015). Moreover, stirred tank bioreactors with impellers achieved a homogenous culture system as it mixes the culture more regularly. As compared with traditional culture using flasks, stirred-tank bioreactors are closed systems that reduce the risk of contamination and with parameter control and monitoring systems which are able to monitor the cultured cells easier (Nienow et al. 2014). According to Mizukami et al. (2017), hollow fibre bioreactor was used in clinical trials testing for intestinal bowel disease (IBD), and successful expansion of up to 11-fold of MSCs in 5 days was reported.

4.3 Preconditioning of MSCs

Preconditioned MSCs under modified culture conditions would help in preserving their therapeutic effects, and this approach has been well studied in stem cell therapy in recent years (Ocansey et al. 2020). For instance, preconditioning of MSCs using IFN-γ seems to be a promising approach in conferring anti-inflammatory effect which was proven by Noone et al. (2013). IFN-γ will specifically target T cells or NK cells and suppress its activity by the release of prostaglandin E2. The suppression of

Table 4 Current conventional treatments (surgical, non-surgical, medical, stem cells) and their cost

Author (year)	Category		Estimated average cost (USD)	No. of patients	Healing rate (%)	Follow-up (weeks)	Complication
van Koperen et al. (2009)	Surgical	Fistulotomy	$2,855	28	82	343	Fistula recurrence (18%)
							Faecal incontinence (61%)
							Total: 79%
Galis-Rozen et al. (2010)		Seton drainage	$2,688	17	59	104	Fistula recurrence (40%)
							Faecal incontinence (6%)
							Total: 46%
Bessi et al. (2019)		Advancement flap	$3,015	34	68	58	Treatment failure (33%)
Gingold et al. (2014)		LIFT	$2,876	15	60	8	Treatment failure (40%)
Senéjoux et al. (2016)		Fistula plug	$2,965	54	31	12	Abscesses formation (7%)
							Plug avulsions (9%)
							CD flare (2%)
							Abdominal pain (2%)
							Miscellaneous (11%)
							Total: 31%
Grimaud et al. (2010)	Non-surgical	Fibrin glue	$2,627	36	38%	8	Abscess formation (11%)
	Medical treatment						
Thia et al. (2009)	Antibiotic	Metronidazole	$9.8 for ten tablets (500 mg) (8)	7	0	10	Abscess (42.9%)
Thia et al. (2009)		Ciprofloxacin	$19 for ten tablets (500 mg) (9)	10	30	10	Abscess (20%)
Bouguen et al. (2013)	Anti-TNF agents	Infliximab	$1,229 (100 mg) (11)	156	69	250	Fistula recurrence (40%)
							Abscess formation (29%)
							Total: 69%
Castaño-Milla et al. (2015)		Adalimumab	$3,120 (40 mg) (13)	46	49	52	Treatment failure (51%)
	Medical treatment						
Panés et al. (2016)	Stem cells	Alofisel (Cx601)	$67,000	107	53	24	TRAEs:
							Anal abscess (6%)
							Proctalgia (5%)
							Procedural pain (1%)
							Fistula discharge (1%)
							Total: 13%
Panés et al. (2017)				107	59	52	TRAEs:
							Anal abscess (13%)
							Proctalgia (5%)
							Procedural pain (1%)
							Total: 19%
Ramezankhani et al. (2020)		Cupistem	$5,000	43	80.8	96	–

immune cells activity will then prevent it from presenting cytotoxicity characteristics (Noone et al. 2013). Also, according to a recent study by Yu et al. (2019), preconditioning of MSCs with IFN-γ and IL-1β upregulates the production of PGE2 and IDO, thus improving the immunomodulatory property of MSCs and increasing its efficacy towards treatment.

Nicotinamide (NAM) can also be used in cell culture to act as a cell supplement. NAM belongs to the family under vitamin B3 and has been used widely for treatment of various diseases such as diabetes, Alzheimer's disease and cancer (Meng et al. 2018). However, studies have shown that using NAM for cell culturing could enhance cells' performance (Meng et al. 2018). This may be due to the role of NAM as a direct effector on ROCK signalling pathway inhibition, thus improving cell survival and inducing cell differentiation (Watanabe et al. 2007; Zhang et al. 2021). Not only that, studies have also proved the role of NAM to inhibit casein kinase 1 (CK1), which involves in CK1 signalling pathway to induce apoptosis (Janovská et al. 2020). Besides this, several studies have proposed that preconditioning of MSCs under hypoxic conditions presented an improvement in proliferation rate. For example, Haque et al. (2013) stated that the proliferation rate and the cell doubling time that cultured for BM-MSCs under hypoxic conditions reported a significant increase as compared to ambient oxygen concentration.

4.4 Genetically Modified MSCs

Genetically modified MSCs have been used widely in recent years in treating various diseases (Varkouhi et al. 2020). Although limited studies are available on using engineered MSCs against PF treatment, there are still several clinical trials reported showing better wound healing rates and improved immunomodulatory effects against certain diseases. These techniques would be useful as a reference to further examine the efficacy of PF treatment. Table 5 summarises the use of genetically modified methods and their effect on immunoregulatory function. Here we have briefly explained the therapeutic benefits of genetically modified MSCs as well as the cost that needs to be taken into consideration before introducing this approach to the clinical.

4.4.1 Improving Migration

The cellular migratory ability is crucial in MSCs treatment where the transplanted or injected MSCs would need to migrate to the injured tissues. The improvement of cell migration can be enhanced by modifying miRNAs. According to the in vitro study in a rat model, overexpression of miR-9-5p would help in upregulating the β-catenin signalling pathway, which takes part in most of the cellular regulatory processes in MSCs, such as differentiation and migration (Li et al. 2017; Pai et al. 2017). Overexpressing miR-9-5p would help to suppress the activity of glycogen synthase kinase 3 beta (G5k3β) and casein kinase 1 alpha (CK1α), which act as inhibitors towards the production of β-catenin. The inactive inhibitors will thus help in preventing the degradation of β-catenin, promote the formation of β-catenin and enhance the signalling pathway at a higher level (Pai et al. 2017). The overall process will thus be promoting sufficient MSCs to migrate towards injured tissues. Modification of miR-9-5p of MSCs in PF treatment may provide similar results with the study mentioned, where further studies need to be conducted to test its efficacy of it.

Enhancement of the cellular migratory process could also be achieved by modifying CXC chemokine receptors, in specific CXCR4 and CXCR7. Based on a study by Devetzi et al. (2018), overexpressing both genes will induce the activity of stromal cell-derived factor-1 (SDF-1) which acts as key chemokines in mediating homing of transplanted MSCs to the injured tissues. This results in promoting paracrine signalling to the nearby cells, thus maximising the efficacy of the wound healing process (Devetzi et al. 2018). According to a study by Du et al. (2013), an animal model has been used to determine the effect of CXCR4 and CXCR7 overexpression in BM-MSCS towards early liver regeneration. The results showed

Table 5 Types of genetic modification process

Author (year)	Genetic modification	Source of MSCs	Experimental model	Transfection method	Transfection efficiency	Purpose	Target mechanism
Du et al. (2013)	Overexpression of CXCR4 and CXCR7	Rat BM-MSCs	Rat with liver injury	Viral (adeno-associated virus)	Approximately 98%	Improves cell migration	Increase level of SDF-1α, VEGF and HGF
Han et al. (2014)	Overexpression of Sox2 and Oct4 gene	Human ADSCs	Human ADSCs	Non-viral (liposomal)	Approximately 98%	Reduces premature senescence of MSCs	Extending S phase in cell cycle by increasing the production of cyclin D1
Li et al. (2017)	Overexpression of miR-9-5p	Rat BM-MSCs	Artificial made wound cells	Non-viral (liposomal)	High	Improves cell migration	Induce β-catenin signalling pathway by suppressing activity of CK1α and G5k3β
Fang et al. (2016)	Exosomal miR-21, miR-23a, miR-125b and miR-145	WJ-MSCs	Mouse model with skin injury	Non-viral (microinjection)	High	Improves wound healing process	Induce the translocation of β-catenin to the injured tissue
Li et al. (2016)	Exosomal miR-181c	UC-MSCs	Mouse model with burn injury	Non-viral (microinjection)	High	Anti-inflammatory	Suppresses macrophage activity to produce IL-6
							Downregulates the secretion of inflammatory cytokines IL-1β and TNF-α
							Increase of IL-10 production
							Inhibits TLR4 expression
Liang et al. (2016)	Overexpression of exosomal miR-125a	Human ADSCs	HUVECs	Non-viral (microinjection)	Approximately 90%	Promotes re-epithelisation	Downregulating the expression of DLL4 thus promotes the formation of epithelial tip cells

Different types of genetic modification approaches of MSCs are described together with its purpose and target mechanism. This may help as a guide to involve genetic modification techniques in PF treatment, where exosomal microRNAs are mostly used

improving cellular migration into rat liver graft, promoting regeneration of hepatocytes in the rat's liver (Du et al. 2013). Although the study was only conducted in animal models, altering CXC chemokine receptors may be a promising technology in PF treatment and should be further examined.

4.4.2 Reduced Premature/Replicative Senescence

Modifying octamer-binding transcription factor 4 (Oct4) and SRY-Box transcription factor 2 (Sox2) genes would help in reducing premature senescence of MSCs. Based on a study by Han et al. (2014), the overexpression of these two genes was transfected in human ADSCs with the help of a PB-CA vector. The findings concluded that overexpressing of Sox2 and Oct4 will prolong the duration of the synthesis (S) phase in the cell cycle, where DNA replication occurs. It is important to monitor the processes in each phase of the cell cycle, as the overall events will eventually affect the cell proliferation performance (Resnitzky et al. 1994). High expression levels of Sox2 and Oct4 will trigger the production of cyclin D1 in high amount, thus accelerating the transition from non-proliferative growth 1 (G1) phase into proliferative S phase, prolonging the duration in DNA replication and resulting in extended MSCs growth and expansion (Han et al. 2014). This may be also included in PF treatment, where the yield of MSCs will be increased and may be suitable in a larger scale of production.

4.4.3 Cost

The cost of genetically modified MSCs is still unknown in disease treatment as it has only been used in smaller-scale experiments, consisting only of phase I or phase II clinical trials (Damasceno et al. 2020). Nevertheless, exosome therapies are still yet to be approved by FDA, which means that all the experimental studies reported are still not being commercialised in the market (An Introduction to Exosome Therapy and Its Costs 2020). Further studies are needed to identify its cost-effectiveness on large-scale production, and the cost should be affordable for every patient, especially in PF treatment.

4.5 Cell-Free Therapy

Cell-free therapy involves the utilisation of exosomes derived from MSCs, and this approach is gaining much interest in recent years mainly in human disease treatment. Several research have shown the potential of involving exosomes derived from MSCs in the wound healing process, which may benefit PF treatment. Facilitation of the wound healing process could be achieved by transfecting WJ-MSCs exosomes into the injured tissues. Fang et al. (2016) stated that WJ-MSCs contain exosomal miR-21, miR-23a, miR-125b and miR-145, and these miRNAs were being proved in promoting myofibroblast and scar formation, accelerating the overall wound healing process. Not only that, treating injured tissues by using WJ-MSCs exosomes improves the re-epithelialising of tissues by promoting translocation of β-catenin into the injured wound to enhance the formation of skin cells and promotes migration (Fang et al. 2016).

Exosomes released from WJ-MSCs would also contribute to the regulation of the immune system by suppressing the activity of macrophages to secrete IL-6, which reduce the inflammatory response (Song et al. 2020). Li et al. (2016) also mentioned that miR-181c-expressed exosomes secreted from WJ-MSCs can downregulate the release of inflammatory cytokines IL-1β and TNF-α while promoting IL-10 production, leading to anti-inflammation of cells. Nevertheless, exosomes derived from ADSCs were reported to have a great contribution towards the re-epithelialisation process. This is due to the presence of overexpressed miR-125a in the exosomes acting as a pro-angiogenic factor, where it encourages the development of endothelial tip cells by lowering the expression level of angiogenic inhibitor delta-like 4 (DLL4) (Liang et al. 2016).

5 Remaining Challenges and Conclusion

Several limitations and challenges remained to be addressed in MSC therapy for PF treatment. In normal circumstances, MSCs will differentiate into fibroblast and myofibroblast in the phase of wound healing, forming scar tissue on the site of injury. However, excessive proliferation of myofibroblast may occur during tissue remodelling, thus causing fibrotic disease or excessive scarring on the remodelled tissue (Darby et al. 2014). Although most of the patients may not be affected, some may feel discomfort or pain at the scarring site (Dwarkasing and Schouten 2013). Regardless of MSC source, the production of stem cells requires GMP compliance to ensure the products are safe to be used for the patients. However, maintaining a lower cost while practising GMP is a crucial, yet challenging, aspect for manufacturing companies. Besides that, implementing a bioreactor system in MSCs production remains unclear. Detailed financial planning should be performed by considering several financial aspects, such as the cost of the bioreactor as well as on the maintenance. On the other hand, the efficacy of using genetically modified MSCs in PF treatment remains uncertain due to limited studies available in the industry. Therefore, further detailed examination and clinical trials would need to be conducted if genetically modified MSCs are to be introduced in treating PF for both its safety and efficacy index. In conclusion, developing affordable PF therapy is important to satisfy the current market demand, especially targeting the Asia Pacific market with the increase of fistulising CD incidence rate in the next 20 years.

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