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EDITORIAL

Application of mesenchymal stem cell therapy for premature ovarian insufficiency: Recent advances from mechanisms to therapeutics

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Abstract

The incidence of premature ovarian insufficiency (POI) is increasing worldwide, particularly among younger women, posing a significant challenge to fertility. In addition to menopausal symptoms, POI leads to several complications that profoundly affect female reproductive function and overall health. Unfortunately, current clinical treatment strategies for this condition are limited and often yield unsatisfactory outcomes. These approaches typically involve hormone replacement therapy combined with psychological support. Recently, mesenchymal stem cell (MSC) therapies for POI have garnered considerable attention in global research. MSCs can restore ovarian reproductive and endocrine functions through diverse mechanisms, including controlling differentiation, promoting angiogenesis, regulating ovarian fibrosis, inhibiting apoptosis, enhancing autocrine and paracrine effects, suppressing inflammation, modulating the immune system, and genetic regulation. This editorial offers a succinct summary of the application of MSC therapy in the context of POI, providing evidence for groundbreaking medical approaches that have potential to enhance reproductive health and overall well-being for women.

Key Words: Mesenchymal stem cell therapy; Mechanism; Premature ovarian insufficiency; Therapeutic; Women

Core Tip: Premature ovarian insufficiency (POI) is an increasing cause of infertility globally, particularly among younger women, with profound effects on reproductive function and health. With limited treatment options and unsatisfactory results, the use of mesenchymal stem cell (MSC) therapies offers promising transformative approaches to restore ovarian function and enhance reproductive health in women. This article provides a concise overview and evidence of the potential benefits of MSC therapy for POI.

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INTRODUCTION

Premature ovarian insufficiency (POI), previously known as premature ovarian failure (POF), is associated with decreased ovarian function in women aged < 40 years, with its prevalence ranging from 1% to 4%[1]. POI not only affects fertility, psychological well-being, and overall quality of life, but it also significantly affects the skeletal, cardiovascular, urogenital, and nervous systems[2]. Its etiology involves a combination of genetic, immunological, environmental, and iatrogenic factors; however, the precise pathogenic mechanisms remain unclear. To date, there is no proven effective strategy that can restore ovarian function, and the current management approaches primarily encompass hormone replacement therapy, fertility management, and psychosocial support[3]. Although these therapies can partially alleviate clinical symptoms associated with POI, they cannot fully restore key aspects of ovarian function, such as hormone secretion, follicular development, and ovulation. In clinical practice, the most commonly employed method involves *in vitro* fertilization and embryo transfer, utilizing donated oocytes from young women[4]. Nonetheless, the application of this technique is limited by constraints related to the oocyte supply and ethical concerns, highlighting the evident need for new and effective treatments in the field of reproductive medicine.

In recent years, there have been significant advancements in the field of regenerative medicine, with stem cells and biomimetic materials emerging as prominent areas of research. Stem cells demonstrate pluripotent differentiation potential and the capacity for indefinite proliferation. These characteristics enable them to repair damaged tissues and enhance organ function, and therefore, they have significant prospects for treating various diseases, through transplantation[5-7]. Stem cells can be categorized based on their source, and they include embryonic and adult stem cells. Adult stem cells, obtained from undifferentiated cells in diverse tissues, such as bone marrow, adipose tissue, and placenta, have advantages that include their abundant availability, low immunogenicity, and ease of isolation and cultivation *in vitro*. Consequently, an increasing body of research suggests that mesenchymal stem cell (MSC) therapy can partially restore ovarian function and preserve fertility in patients affected by infertility[8-10], presenting a novel avenue for regenerative therapy with immense clinical potential. Therefore, advancing our comprehension of the underlying mechanisms of POI will facilitate further exploration in the field of reproductive medicine and guide the prudent application of MSCs for the treatment of infertility.

BONE MARROW MSCS

Bone marrow MSCs (BMSCs) are adult stem cells derived from bone marrow with noteworthy biological characteristics, such as pluripotent differentiation capacity across germ layers and multipotent differentiation potential. In recent years, several clinical studies have been conducted involving autologous BMSC transplantation based on more than 40 patients with POF. Remarkably, three patients successfully regained normal menstrual cycles, and two achieved pregnancy[11,12]. Igboeli *et al*[13] documented two Caucasian patients with POF who exhibited restored ovarian hormone secretion, improved menstrual cycles, and alleviated menopausal symptoms after laparoscopic autologous BMSC transplantation into the ovaries. Clinical studies have also provided promising evidence for the utility of BMSC transplantation for patients with POF, to achieve successful pregnancies.

Notably, the role of BMSCs in restoring ovarian function has been validated using multiple animal models. The underlying mechanisms involve the suppression of inflammatory responses, inhibition of granulosa cell (GC) apoptosis, attenuation of ovarian tissue fibrosis, promotion of angiogenesis, and differentiation into GCs[14,15]. Early studies indicated that BMSCs are primarily located in the ovarian hilum and medulla, suggesting their potential involvement through paracrine actions[14,15]. Further investigations by Gabr *et al*[16] demonstrated that insulin-like growth factor-1 (IGF-1) and tumor necrosis factor-α induce BMSC homing *in vivo*. Moreover, Bao *et al*[17] and Park *et al*[18] reported that mice with POF who received BMSC treatment exhibit increased numbers of ovarian follicles at various stages, elevated sex hormone levels, and the restoration of ovarian reserves and fertility. Additionally, BMSCs significantly downregulate the mRNA expression of p21, BAX, and c-myc, thereby reducing GC apoptosis. Further, Fu *et al*[19] demonstrated that miR-21 overexpression in BMSCs enhances ovarian structure and function, which are impaired in a chemotherapy-induced POF rat model. This effect was found to be achieved through downregulation of the expression of phosphatase and tensin homolog and programmed cell death protein 4 genes, subsequently inhibiting GC apoptosis[19]. The latest

systematic review summarized the potential therapeutic mechanisms through which BMSCs can ameliorate POF. These mechanisms include homing, angiogenesis, anti-apoptosis, anti-inflammatory and immune regulation, paracrine signaling, mitochondrial transfer, autophagy, and anti-fibrosis and antioxidative effects[20].

ADIPOSE-DERIVED MSCS

Adipose tissue serves as a readily accessible source of stem cells with remarkable proliferative, differentiation, and immunoregulatory capacities. Accumulating evidence has demonstrated the anti-inflammatory, antioxidative, immunoregulatory, angiogenic, and regenerative properties of adipose-derived MSCs (ADMSCs). Studies have noted disturbances in the proportions of peripheral blood lymphocyte subsets in women with POF, including a decrease in the CD4+/CD8+ cell ratio[21]. ADMSCs can increase levels of transforming growth factor-beta1 and interleukin-10 in serum, resulting in expansion of the regulatory T cell population, thereby regulating immune functions and restoring ovarian function in POF[22]. Furthermore, ADMSC transplantation exerts an anti-apoptotic effect by modulating connexin 43 and pannexin 1 during the treatment of POI[23]. Co-culturing the extracellular vesicles of ADMSCs with GCs from women with POI has been found to promote cell proliferation, downregulate suppressor of mothers against decapentaplegic family protein expression, and inhibit the expression of genes associated with GC apoptosis[24]. Moreover, Ding *et al*[25] revealed that ADMSCs can activate the silent mating type information regulation 1 and forkhead box O1 (FOXO1) signaling pathway through the secretion of hepatocyte growth factor (HGF) and basic fibroblast growth factor (FGF), thereby alleviating oxidative stress injuries and restoring ovarian function in mice. In addition, Qu *et al*[26] demonstrated that ADMSC administration, through tail-vein injection, is a potential method to promote the restoration of chemotherapy-induced POF. This approach helps to attenuate apoptosis and senescence in ovarian GCs.

UMBILICAL CORD MSCS

Umbilical cord MSCs (UCMSCs) represent an additional promising source for stem cell transplantation therapy for POI. Clinical research conducted by Ding *et al*[27] demonstrated that UCMSCs activate primordial follicles by phosphorylating FOXO3 and FOXO1 proteins. Another clinical study reported successful births from four patients with POI after UCMSC transplantation, with a shorter duration of amenorrhea yielding more favorable outcomes[28]. The reparative effects of UCMSCs on the ovary have been observed using models of chemotherapy-induced injuries and natural aging[29]. Research has indicated that UCMSCs can restore the ovarian structure and function in mice with POI by regulating the Th1/Th2 cytokine ratio and the number of natural killer cells[30]. UCMSCs express heme oxygenase-1 and can improve ovarian functions in POI mice by modulating the autophagy pathway through activation of the c-Jun N-terminal kinase/B-cell lymphoma 2 signaling pathway and the circulation of CD8+CD28- T cells[31]. Furthermore, studies have evidenced that UCMSC transplantation results in the downregulation of superoxide dismutase and uncoupling protein 2 expression, suggesting potential mechanisms involving a reduction in oxidative stress and the mitigation of ovarian damage[32]. Additionally, Sun *et al*[33] reported that extracellular vesicles derived from UCMSCs inhibit chemotherapy drug-induced stress and apoptosis in ovarian GCs. These findings provide valuable insights for the future clinical application of UCMSCs for the treatment of POI. Moreover, Luo *et al*[34] discovered that therapy using human UCMSCs can restore ovarian functions in animals with POI by inhibiting the apoptosis of theca interstitial cells through the regulation of NR4A1-mediated mitochondrial mechanisms.

MENSTRUAL BLOOD-DERIVED MSCS

Menstrual blood-derived MSCs (MenSCs) offer several advantages, including their abundant sources, non-invasive acquisition, and low immunogenicity. As evidenced by a recent clinical trial involving 15 patients with POF, the intraovarian administration of MenSCs improved ovarian function and led to the restoration of menstrual cycles[35]. Research has also demonstrated that MenSCs can express multiple cell factors, such as HGF, IGF-1, and FGF-2, which promote GC maturation and differentiation *in vitro*. These cell factors play a significant role in ovarian repair[36]. Yamchi *et al*[37] reported that MenSC transplantation could modulate the expression levels of fibrosis-related genes, potentially restoring the structure and functions of damaged ovaries. Moreover, Fu *et al*[38] demonstrated that MenSC transplantation could improve the ovarian microenvironment by reducing GC apoptosis and ovarian stromal fibrosis. Further, Zhang *et al*[39] found that *in vivo*, the transplantation of extracellular vesicles derived from MenSCs promotes follicle development, restores estrous cycles and hormone levels, and improves pregnancy outcomes in rats with POI. Additionally, MenSCs were found to regulate the ovarian extracellular matrix composition, facilitate the recruitment of dormant follicles within the ovarian cortex, and enhance GC proliferation within follicles[39]. In conclusion, MenSCs present a promising and effective approach for the treatment of POI.

LIMITATIONS AND CHALLENGES ASSOCIATED WITH STEM CELL THERAPY FOR POI

Although the efficacy of stem cell transplantation has been extensively demonstrated based on numerous animal models, its clinical application remains relatively scarce. The methods and specifics of stem cell transplantation are still in the exploratory stage. Therefore, safety assessments for stem cell therapy remain a primary concern for infertility treatments for patients with POI. This is because when certain stem cells are transplanted into the body, they could lose their characteristic features, such as their high proliferation and differentiation capacity, and there is a chance of epigenetic modifications and chromosomal mutations, which can pose various risks. Moreover, their limited sources, associated ethical controversies, low survival rates of implanted cells, immune responses, and risks of tumor formation associated with stem cell transplantation add to these challenges. In recent years, research on the paracrine effects of stem cells has gained attention, and the concept of cell-free therapy for biologic treatments has emerged. Stem cell-derived extracellular vesicles, such as exosomes, have shown promise as a novel cell-free therapy for treating POI. For instance, Qu *et al*[26] reported that extracellular vesicles derived from human UCMSCs promote ovarian angiogenesis and inhibit ovarian GC apoptosis in a cisplatin-induced POF rat model through the delivery of miR-126-3p. Recently, a novel exosome-encapsulated microcarrier, prepared using microfluidic technology, was also presented for ovarian repair after chemotherapy-induced damage[40]. However, literature on cell-free therapy for infertility is currently limited, and further preclinical exploration is needed. Extensive experimental studies are crucial to explore the mechanisms underlying the effects of stem cell therapy for preserving female reproductive health. Large-scale clinical trials are also essential for ascertaining the safety and efficacy of stem cell therapy, with the goal of establishing a canonical consensus for future treatments.

CONCLUSION

We have provided a comprehensive overview of the application of different MSC types for the treatment of POI and have elucidated their potential mechanisms. The ultimate goal is to develop regenerative medicine and biomedical engineering strategies that can effectively cure POI. Although there are certain limitations to consider, a thorough understanding of the current research evidence is crucial for reproductive assistance agencies to formulate future translational applications and clinical trial guidelines for stem cell therapies for female infertility.

FOOTNOTES

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EDITORIAL

Use of priming strategies to advance the clinical application of mesenchymal stromal/stem cell-based therapy

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Abstract

Mesenchymal stromal/stem cells (MSCs) have garnered significant attention in the field of regenerative medicine due to their remarkable therapeutic potential. MSCs play a pivotal role in maintaining tissue homeostasis and possess diverse functions in tissue repair and recovery in various organs. These cells are characterized by easy accessibility, few ethical concerns, and adaptability to *in vitro* cultures, making them a valuable resource for cell therapy in several clinical conditions. Over the years, it has been shown that the true therapeutic power of MSCs lies not in cell engraftment and replacement but in their ability to produce critical paracrine factors, including cytokines, growth factors, and exosomes (EXOs), which modulate the tissue microenvironment and facilitate repair and regeneration processes. Consequently, MSC-derived products, such as conditioned media and EXOs, are now being extensively evaluated for their potential medical applications, offering advantages over the long-term use of whole MSCs. However, the efficacy of MSC-based treatments varies in clinical trials due to both intrinsic differences resulting from the choice of diverse cell sources and non-standardized production methods. To address these concerns and to enhance MSC therapeutic potential, researchers have explored many priming strategies, including exposure to inflammatory molecules, hypoxic conditions, and three-dimensional culture techniques. These approaches have optimized MSC secretion of functional factors, empowering them with enhanced immunomodulatory, angiogenic, and regenerative properties tailored to specific medical conditions. In fact, various priming strategies show promise in the treatment of numerous diseases, from immune-related disorders to acute injuries and cancer. Currently, in order to exploit the full therapeutic potential of MSC therapy, the most important challenge is to optimize the modulation of MSCs to obtain adapted cell therapy for specific clinical disorders. In other words, to unlock the complete potential of MSCs in regenerative medicine, it is crucial to identify the most suitable tissue source and develop *in vitro* manipulation protocols specific to the type of disease being treated.

Key Words: Mesenchymal stromal/stem cells; Therapeutic properties; Paracrine effects; Cell priming; Cell-free therapies; Regenerative medicine

Core Tip: Mesenchymal stromal/stem cells (MSCs) offer important therapeutic effects in the field of regenerative medicine. Their key role lies in the production of paracrine factors that modulate tissue environments and allow their repair following insults. Recently, MSC-derived products such as exosomes and conditioned media are replacing whole MSCs in clinical applications. In this regard, to optimize the results of MSC-based treatment, researchers have explored priming strategies in order to enhance MSC properties. Realizing the full potential of MSC therapy depends on identifying the right tissue source and developing priming strategies specific to the disease being treated.

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INTRODUCTION

Over the years, mesenchymal stromal/stem cells (MSCs) have emerged as an important therapeutic tool in the field of regenerative medicine[1-4]. These versatile multipotent adult stromal/stem cells play a crucial role in maintaining tissue homeostasis under both physiological and pathological conditions. In fact, MSCs possess the remarkable ability to influence their surroundings by differentiating, attracting supporting cells, and orchestrating central processes for tissue regeneration[5,6]. Together, the multifaceted potential of MSCs shed light on their role as key regulatory elements in the complex mechanisms governing tissue repair/recovery in several tissues, including the intestine[7], skin[8], and skeletal muscle[6], where MSCs exhibit diverse functions, either supporting high cellular turnover or facilitating regeneration following injury.

These discoveries offer a strong motivation for investigating the potential of MSCs as a cellular therapeutic product to enhance tissue injury responses in various diseases[9-14]. MSCs show high accessibility, minimal ethics-related concerns, and great adaptability to *in vitro* cultures for expansion[15]. Moreover, these cells possess immune privilege attributed to their low expression of CD40, CD80, CD86, and major histocompatibility complex I (MHC I), along with the absence of MHC II expression[16,17]. These attributes make these cells a highly valuable resource for developing new cell therapies in the field of regenerative medicine.

MSCs are present in various tissues, including bone marrow[18], adipose tissue[19], umbilical cord[14], dental pulp [20], and placenta[21]. In these diverse tissue environments, MSCs interact with different cell types, such as epithelial cells, endothelial cells, immune cells, and stromal cells, showing immunomodulatory, angiogenic, pro-trophic, and anti-oxidative properties[22-25]. Their adaptability and therapeutic potential make them promising candidates for addressing a wide range of clinical disorders, including cardiovascular, neurodegenerative, immune, lung, liver, kidney, and orthopedic diseases. Notably, it has become increasingly evident that the true therapeutic power of MSC therapies lies not in engraftment and cell replacement but rather in their ability to produce critical paracrine factors that modulate the tissue microenvironment and facilitate repair and regeneration processes. Indeed, these cells are able to produce crucial functional factors, such as cytokines, growth factors, and exosomes (EXOs), which can mediate their therapeutic effects [26-28]. Hence, given the regenerative potential and trophic properties inherent in certain MSC-derived products, such as the conditioned medium and/or EXOs, these products have arisen as potential therapeutic tools with a wide range of applications. Consequently, they are undergoing extensive evaluation for potential medical use[9,12,29-32]. The clinical utilization of MSC-derived products must be considered for their advantages, particularly in contrast to concerns related to the prolonged use of MSCs and the associated risks of infectious disease transmission, such as viruses present in transplanted allogeneic cells[33].

However, the therapeutic landscape of MSCs is not without its challenges and controversies. The efficacy of MSC-based treatments has yielded variable results in clinical trials, reflecting the complexity of intrinsic differences between cell-based products and a lack of standardized methods for MSC production that affects their potency[34-39]. The effects of MSCs vary based on the tissue source and the methods employed in their production and administration[35,40,41]. Several studies have demonstrated that the composition of the MSC secretome can be modulated through the preconditioning of MSCs with cytokine treatments and hypoxia. Additionally, cultivating MSCs under specific culture systems, such as three-dimensional (3D) conditions, also influences their secretome. In response to MSC "priming", the production of factors is switched towards a greater functional phenotype that results in an increase in MSC therapeutic effects[3,27,42].

The field of research on MSCs is still very complex and is constantly evolving, emphasizing that the road to consolidating the use of MSCs as an effective cell therapy for various pathologies is still quite long. In this regard, promising approaches are being studied, among which MSC priming certainly represents one of the most hopeful strategies.

PRIMING STRATEGIES TO POTENTIATE THE THERAPEUTIC EFFECTS OF MSCs

In the last decade, the concept of priming or preconditioning MSCs has gained credibility as a means to enhance MSC therapeutic potential by modulating the secretion of paracrine factors and tailoring their actions to specific medical conditions[3,27]. Similar to immune cells[43], MSCs have been shown to memorize a stimulus after transitioning to a new environment[44]. In this regard, MSCs can be primed to generate a short-term-memory effect and, mimicking microenvironmental stimuli, this strategy may be used *in vitro* to avoid the need for *in vivo* activation of the MSCs when aiming towards specific therapeutic activities. This approach has been widely explored in the context of immunomodulation[45,46], tissue regeneration[47,48], and even cancer interactions[49], with each priming strategy offering a unique set of advantages and applications.

One of the principal priming strategies involves exposing MSCs to inflammatory molecules. Numerous studies reveal that the immunosuppressive properties of MSCs are not intrinsic but require priming by inflammatory factors. In fact, depending on the specific inflammatory conditions, the MSC phenotype can be polarized into MSC type 1, characterized by pro-inflammatory properties, or MSC type 2, with immunosuppressive capabilities[50]. Various strategies have been implemented to modulate and enhance the secretion of immunomodulatory molecules in MSCs. The treatment of MSCs with inflammatory cytokines, including interferon-γ, interleukin (IL)-1α/β, IL-6, tumor necrosis factor (TNF)-α, and IL-17, is shown to significantly enhance their immunomodulatory properties. This priming approach increases the production and secretion of key functional factors such as hepatocyte growth factor (HGF), transforming growth factor (TGF)-β, IL-6, prostaglandin E2 (PGE2), leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor, IL-10, macrophage inflammatory protein (MIP)-1α, indoleamine 2,3-dioxygenase (IDO), intercellular adhesion molecule, programmed death ligand (PDL)1-2, monocyte chemoattractant protein (MCP)-1, monokine induced by interferon-gamma, interferon-gamma-inducible protein 10, and MIP-1β. These factors, in turn, empower MSCs with enhanced paracrine immunomodulatory properties, making them potent inhibitors of T cell proliferation and activators of anti-inflammatory M2 macrophage polarization[27]. Moreover, treatment with inflammatory cytokines is shown to improve the immunomodulatory capabilities of extracellular vesicles (EVs) derived from MSCs, further highlighting the versatility of this priming strategy in the context of immunoregulation[45,51].

Priming with hypoxia represents another pivotal approach to enhancing MSC functionality. Hypoxic preconditioning of MSCs is shown to stimulate the secretion of essential growth factors, such as vascular endothelial growth factor (VEGF) and HGF, which are crucial for angiogenesis and tissue regeneration[52]. Under hypoxic conditions, MSCs activate signaling pathways, including the HIF-1α-GRP78-Akt axis, leading to the overproduction of pro-angiogenic factors[53]. This approach yields significant benefits in various acute injuries, including ischemia-reperfusion injury (IRI), renal injury, and myocardial infarction[3]. Moreover, hypoxic preconditioning is effective in promoting hepatic tissue regeneration, with increased expression of factors such as HGF and VEGF[48,54]. This is particularly advantageous in cases of liver injury and fibrosis. Hypoxic MSCs also exhibit the ability to secrete functional EVs capable of stimulating tissue remodeling, contributing to tissue repair in cerebral tissue[55]. In addition, hypoxic MSC-derived EVs show enhanced activity both *in vitro* and *in vivo*, especially in promoting angiogenesis on human brain microvascular endothelial cells. Interestingly, this effect appears to be mediated by microRNA (miRNA)-612[56]. Therefore, several functional factors produced by hypoxia-primed MSCs are found to play a crucial role in stimulating angiogenic and regenerative activities, making this priming strategy a valuable tool to enhance MSC therapeutic effects for tissue recovery after acute injury.

Priming through 3D culture techniques offers an alternative approach to enhancing MSC therapeutic properties. This strategy involves the generation of MSC spheroids, which closely mimic the *in vivo* MSC niche and boost the functional phenotypic profile of MSCs. These spheroids exhibit superior trophic and immunomodulatory functionalities, driven by the paracrine secretion of functional factors with anti-inflammatory, angiogenic, anti-fibrotic, anti-apoptotic, and mitogenic properties[30,51,57-59]. Comparative studies show that 3D culture of MSCs can modify their transcriptome profile, leading to the overexpression of genes that regulate proliferation, differentiation, immunomodulation, and angiogenic processes[60]. These spheroids are found to secrete a plethora of regenerative and immunomodulatory factors, including stromal cell-derived factor-1α, growth-regulated oncogene α, MCP-1/3, IL-4, IL-10, EGF, LIF, placental growth factor-1, VEGF-A/D, HGF, insulin-like growth factor 1, TNFAIP6, stanniocalcin 1, PDGFB, TGF-β, PGE2, and IDO. Such factors are involved in promoting tissue repair and regeneration, making 3D-cultured MSCs valuable for various applications in regenerative medicine[27].

PRIMING STRATEGIES TO IMPROVE THE CLINICAL APPLICATION OF MSCs

The application of these priming strategies is not limited to basic research. They have found practical utility in the treatment of various clinical conditions (Table 1). For instance, in the context of chronic immune-related disorders, MSCs primed with pro-inflammatory cytokines demonstrate enhanced immunomodulatory properties, making them more effective in diseases such as colitis, autoimmune encephalomyelitis, and graft-*versus*-host disease (GVHD)[61,66,102]. Notably, the priming of MSCs with IL-1β shows promise in alleviating the side effects of sepsis, primarily by inducing macrophage polarization toward an anti-inflammatory M2 phenotype[103]. Similarly, the use of TNF-α-primed MSCs attenuates symptoms of GVHD and peritonitis, with a demonstrated reduction in pro-inflammatory cytokines and an increase in anti-inflammatory factors[67]. Moreover, the efficacy of MSCs primed with 3D culture conditions is evident in the treatment of diseases characterized by unresolved inflammation, as these spheroids overexpress TSG-6 and exhibit a more significant impact in reducing inflammation[92].

Table 1 Main priming strategies of mesenchymal stromal/stem cells and their application in various disease models

MSCs	Priming treatments	Model/disease	Therapeutic effects	Ref.
Priming with inflammatory molecules				
BM-MSCs	IFN-γ	*In vivo* model of chronic colitis	Attenuation of inflammation	[61]
UC-MSCs	TNF-α	*In vivo* model of intrauterine adhesion	Reduction of inflammation and endometrium fibrosis	[62]
BM-MSCs	IFN-γ	*In vivo* models of acute radiation syndrome	Protection from radiation-induced lethality	[63]
UC-MSCs	IL-1β	*In vivo* model of chronic colitis	Attenuation of inflammation	[64]
BM-MSCs	IL-25	*In vivo* model of chronic colitis	Attenuation of inflammation	[65]
BM-MSCs and CB-MSCs	IFN-γ	*In vivo* model of GVHD	Reduction of the symptoms of GVHD	[66]
UC-MSCs	IFN-γ; TNF-α	*In vivo* model of GVHD	Reduction of the clinical symptoms	[67]
BM-MSCs	IL-6	*In vivo* model of liver fibrosis	Reduction of liver injury	[68]
UC-MSCs	IL-1β	*In vivo* model of sepsis	Increase in survival rate	[69]
CB-MSCs	IFN-γ	*In vivo* model of acute kidney injury	Reduction of kidney injury	[70]
AdMSCs	TNF-α	*In vivo* model of wound healing	Acceleration of wound closure and angiogenesis	[71]
Priming with hypoxia				
BM-MSCs	Hypoxia	*In vivo* model of traumatic brain injury	Improved neurogenesis and cognitive function	[47]
AdMSCs	Hypoxia	*In vivo* model of hepatectomy	Enhanced liver regeneration	[48]
UC-MSCs	Hypoxia	*In vivo* model of spinal cord injury	Improved axonal preservation	[52]
AdMSCs	Hypoxia	*In vivo* model of hindlimb ischemia	Improvement of angiogenesis	[53]
BM-MSCs	Hypoxia	*In vivo* model of hepatectomy	Enhanced liver regeneration	[54]
BM-MSCs	Hypoxia	*In vivo* model of pulmonary fibrosis	Increased survival rate	[72]
BM-MSCs	Hypoxia	*In vivo* model of hindlimb ischemia	Improvement of angiogenesis	[73]
AdMSCs	Hypoxia	*In vivo* model of hindlimb ischemia	Improvement of functional recovery	[74]
BM-MSCs	Hypoxia	*In vivo* model of radiation-induced lung injury	Improvement of antioxidant ability	[75]
BM-MSCs	Hypoxia	*In vivo* model of lung IRI	Attenuation of lung injury	[76]
AdMSCs	Hypoxia	*In vivo* model of acute kidney injury	Improvement of renal function	[77]
AdMSCs	Hypoxia	*In vivo* model of acute kidney injury	Attenuation of kidney injury	[78]
PMSCs	Hypoxia	*In vivo* model of scar formation	Reduction of scar formation	[79]
AF-MSCs	Hypoxia	*In vivo* model of wound healing	Acceleration of wound healing	[80]
BM-MSCs	Hypoxia	*In vivo* model of wound healing	Acceleration of wound healing	[81]
BM-MSCs	Hypoxia	*In vivo* model of hindlimb ischemia	Improvement of muscle fiber regeneration	[82]
DP-MSCs	Hypoxia	*In vivo* model of dental pulp injury	Regeneration of dental pulp	[83]
BM-MSCs	Hypoxia	*In vivo* model of cerebral ischemia	Enhanced angiogenesis and neurogenesis	[84]

BM-MSCs	Hypoxia	*In vivo* model of ischemic cortex	Reduction of infarct volume	[85]
BM-MSCs	Hypoxia	*In vivo* model of myocardial infarction	Reduction of cardiac fibrosis	[86]
BM-MSCs	Hypoxia	*In vivo* model of myocardial infarction	Improvement cardiac functions	[87]
BM-MSCs	Hypoxia	*In vivo* model of myocardial infarction	Prevention of apoptosis in cardiomyocytes	[88]
BM-MSCs	Hypoxia	*In vivo* model of myocardial infarction	Increased cardiomyocyte proliferation and function	[89]
BM-MSCs	Hypoxia	*In vivo* model of myocardial infarction	Improved cardiac repair	[90]
BM-MSCs	Hypoxia	*In vivo* IRI model of myocardium	Reduction of IRI	[91]
Priming with 3D culture				
BM-MSCs	3D culture	*In vivo* model of peritonitis	Attenuation of inflammation	[92]
UC-MSCs	3D culture	*In vivo* model of arthritis	Attenuation of systemic arthritic manifestations	[93]
CB-MSCs	3D culture	*In vivo* model of hindlimb ischemia	Improvement of cell survival and angiogenesis	[94]
AdMSCs	3D culture	*In vivo* model of hindlimb ischemia	Improvement of angiogenesis	[95]
AdMSCs	3D culture	*In vivo* model of acute kidney injury	Amelioration of renal function	[96]
AdMSCs	3D culture	*In vivo* model of disc degeneration	Induction of disc repair	[97]
BM-MSCs	3D culture	*In vivo* model of bilateral calvarial defects	Induction of bone regeneration	[98]
SMSCs	3D cultures	*In vivo* model of osteochondral defects	Induction of cartilage regeneration	[99]
BM-MSCs	3D culture	*In vivo* model of myocardial infarction	Promotion of cardiac repair	[100]
BM-MSCs	3D cultures	*In vivo* model of myocardial infarction	Improvement of cardiac function	[101]

MSCs: Mesenchymal stromal/stem cells; BM-MSCs: Bone marrow-derived mesenchymal stromal/stem cells; UC-MSCs: Umbilical cord-derived mesenchymal stromal/stem cells; AdMSCs: Adipose-derived mesenchymal stromal/stem cells; CB-MSCs: Cord blood-derived mesenchymal stromal/stem cells; DP-MSCs: Dental pulp-derived mesenchymal stromal/stem cells; PMSCs: Placenta-derived mesenchymal stem cells; AF-MSCs: Amniotic fluid derived mesenchymal stromal/stem cells; SMSCs: Synovial derived mesenchymal stromal/stem cells; GVHD: Graft-*versus*-host disease; IRI: Ischemia-reperfusion injury; IFN: Interferon; TNF: Tumor necrosis factor; IL: Interleukin; 3D: Three-dimensional.

The therapeutic potential of MSCs also extends to the treatment of acute injuries, where priming strategies can play a crucial role in boosting their regenerative capabilities. For instance, in cases of acute myocardial injury, hypoxic preconditioning significantly improves blood flow recovery, influences heart remodeling, and enhances the regeneration of ischemic tissues[87,88]. These effects are attributed to the increased production of pro-survival and pro-angiogenic factors by hypoxia-primed MSCs, including HIF-1α, ANGPT1, VEGF, Flk-1, Bcl-2, and Bcl-xL[87]. Hypoxic MSCs demonstrate enhanced integration into damaged tissues, with improved survival, proliferation, and regenerative effects[74]. In parallel, 3D-cultured MSCs show potential in both bone and cartilage repair, highlighting their capacity to stimulate tissue regeneration across various contexts[98,99].

In recent years, the interaction between MSCs and cancer has also garnered considerable attention. Indeed, MSCs represent a crucial actor in the tumor microenvironment due to their ability to modulate the function/survival of both immune cells and tumor cells, with the final effects of promoting or inhibiting cancer[104]. Numerous studies have investigated the molecular mechanisms involved in the MSC-based modulation of tumor immunity, revealing that MSCs might either support or suppress tumor progression since many MSC factors can be produced differently in the tumor microenvironment[104-106]. For instance, the cross-talk between MSCs and M1/M2 macrophages plays a pivotal role in regulating tumor progression[107]. MSCs are shown to promote the shift from anti-tumorigenic M1 macrophages to pro-tumorigenic M2 macrophages, contributing to immune evasion and tumor growth[108]. Moreover, the capacity of MSCs to express immune checkpoint molecules, including PDL1, further intensifies their role in immunosuppression, facilitating the evasion of host immune responses by cancer cells[109]. On the other hand, various studies indicate that utilizing MSC-derived EVs housing anti-tumorigenic miRNAs might offer a novel therapeutic opportunity for MSC-based tumor therapy[110].

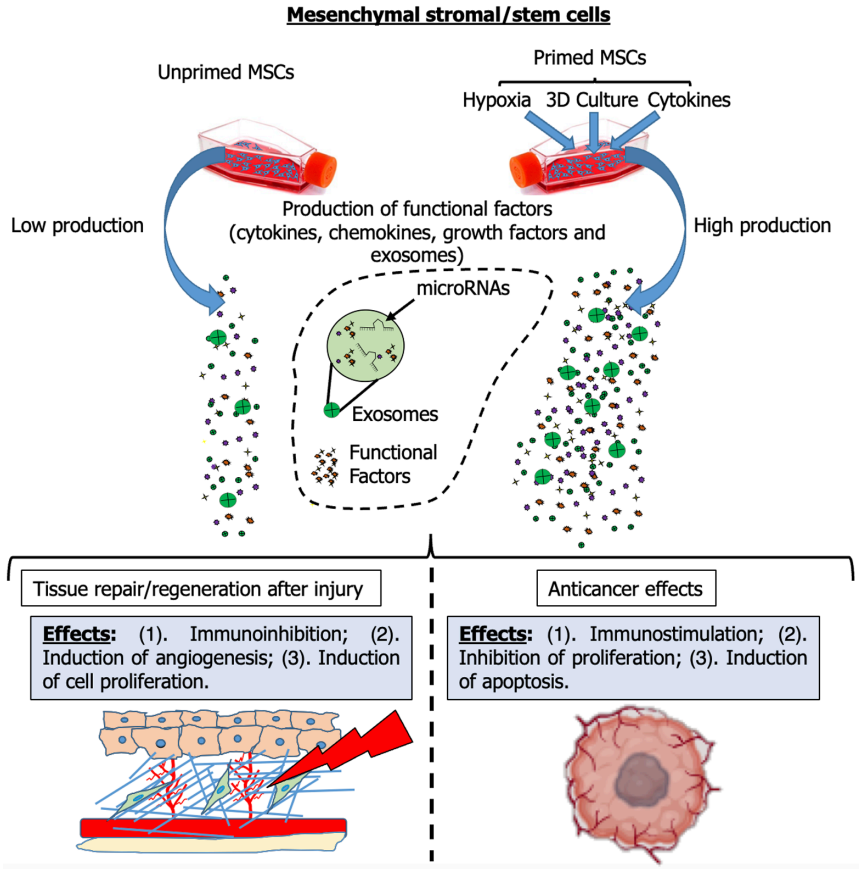

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Figure 1 Schematic representation of the enhanced therapeutic effects of mesenchymal stromal/stem cells after priming. Mesenchymal stromal/stem cells can be activated through various stimuli to increase the production of functional factors. Depending on the type of priming employed, different effects can be achieved, such as immunoinhibition, induction of angiogenesis, and cellular proliferation, which can be exploited for tissue repair/regeneration following injury. Conversely, when different effects are induced by diverse priming strategies, such as immunostimulation, inhibition of proliferation, and induction of apoptosis, these effects can be harnessed for anticancer treatment. MSC: Mesenchymal stromal/stem cell.

In summary, priming strategies represent a versatile approach to managing the therapeutic potential of MSCs, tailoring their secreted factors and interactions to diverse clinical conditions. These strategies show great promise in regenerative medicine, immune-related disorders, and the complex interplay between MSCs and cancer (Figure 1). Through exposure to inflammatory molecules, hypoxic environments, 3D culture conditions, or other new priming strategies, MSCs can be transformed into highly specialized therapeutic tools, extending the possibilities for their application in various clinical settings and expanding our understanding of the dynamic role of MSCs in health and disease. The ongoing research in this field promises further advancements in the optimization of MSC-based therapies, offering new hope for patients suffering from a wide range of pathologies.

DISCUSSION

While research on MSCs is booming, as are their clinical applications, it is becoming increasingly important to understand the multiple properties of MSCs and how these can be optimally modulated to achieve the desired therapeutic effects. The use of MSC therapy, unfortunately, suffers from intrinsic biological variability, both due to the source and inter-subject variability. On the other hand, these therapies might prove to be decisive in the treatment of certain so-called multifactorial pathologies where multiple molecular targets are involved, as in the case of inflammatory-related diseases [111], including Alzheimer's and Parkinson's diseases[112,113], cancer[114], IRI[13,115], and others. Due to the ability of MSCs to produce multiple functional factors capable of acting simultaneously on multiple targets, cell therapies based on the use of MSCs might be successful in the treatment of some such acute and chronic diseases for which effective treatments are currently lacking (Figure 2).

However, to achieve this goal, it will be necessary to understand how to modulate MSCs according to the specific dysfunction to be treated. In fact, while MSC immune inhibitory and pro-angiogenic effects may be suitable for various diseases in the field of regenerative medicine, the same properties might be disadvantageous in the treatment of some tumors. In the case of immune-mediated diseases such as GVHD or liver cirrhosis, MSCs with pronounced immunomodulatory capabilities might show enhanced therapeutic efficacy. Also, in the context of wound healing, MSCs displaying a well-balanced array of therapeutic attributes, encompassing immunomodulation, trophic stimulation, and angiogenic

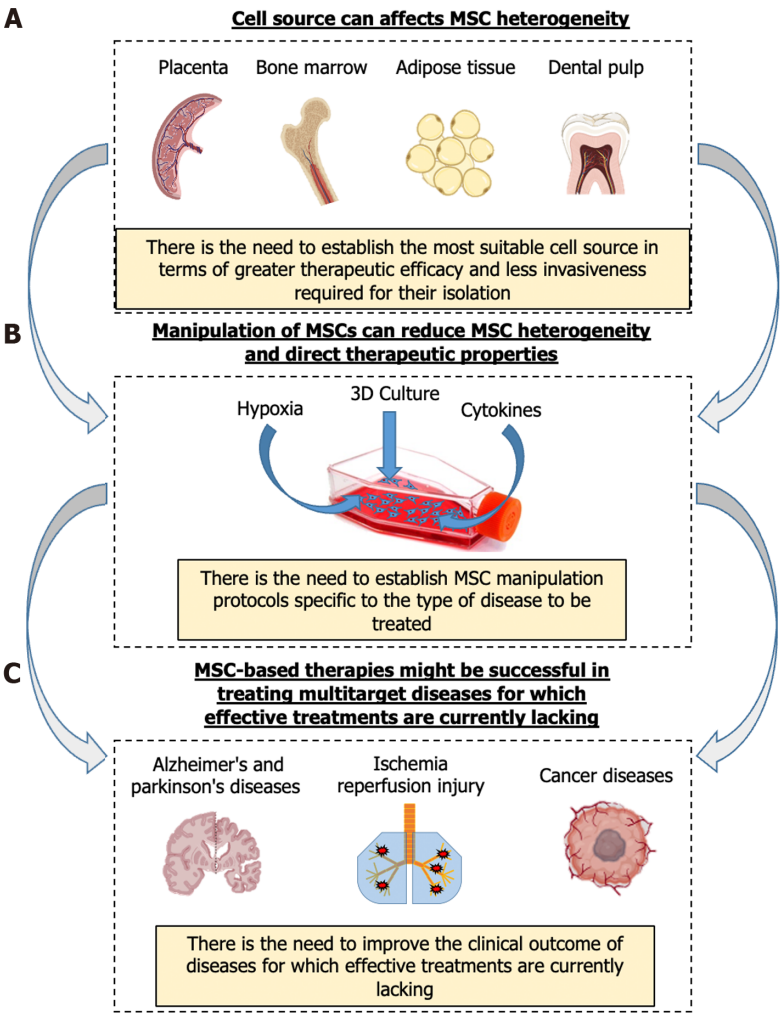

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Figure 2 Important factors affecting the heterogeneity of mesenchymal stromal/stem cell clinical effects and potential strategies for improving mesenchymal stromal/stem cells-based therapies. A: Mesenchymal stromal/stem cells (MSCs) can be isolated from many tissues but have mainly been harvested from bone marrow, dental pulp, adipose and placental tissue. Different tissue sources can affect the MSC phenotype and properties[41]. There is the need to establish the best source for MSCs to obtain, without invasiveness, effective cells for therapeutic use; B: The manipulation of MSCs prior to use can influence MSC clinical potency[3] and direct their use towards specific pathological conditions; C: The above-mentioned strategies might be very useful for the optimization of MSC-based therapies for several multitarget diseases for which effective treatments are currently lacking. MSC: Mesenchymal stromal/stem cell.

promotion, may be more efficacious.

CONCLUSION

It is true that MSCs from various sources possess unique therapeutic properties, but it is unthinkable that they can be extracted from any tissue and used as they are for various types of diseases. The only way to build an effective cell therapy based on MSCs is to first establish the most suitable source in terms of therapeutic efficacy with the least invasive strategy required for their isolation. Subsequently, appropriate *in vitro* manipulation strategies should be studied to promote their expansion and trigger specific therapeutic functions in order to establish MSC manipulation protocols specific to the type of disease to be treated. Our future goal should be to unlock the full potential of MSCs, fostering a deeper appreciation of their remarkable therapeutic capabilities and actively contributing to the ongoing progress of regenerative medicine.

FOOTNOTES

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Effects of exosomes from mesenchymal stem cells on functional recovery of a patient with total radial nerve injury: A pilot study

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Abstract

BACKGROUND

Peripheral nerve injury can result in significant clinical complications that have uncertain prognoses. Currently, there is a lack of effective pharmacological interventions for nerve damage, despite the existence of several small compounds,

peptides, hormones, and growth factors that have been suggested as potential enhancers of neuron regeneration. Despite the objective of achieving full functional restoration by surgical intervention, the persistent challenge of inadequate functional recovery remains a significant concern in the context of peripheral nerve injuries.

AIM

To examine the impact of exosomes on the process of functional recovery following a complete radial nerve damage.

METHODS

A male individual, aged 24, who is right-hand dominant and an immigrant, arrived with an injury caused by a knife assault. The cut is located on the left arm, specifically below the elbow. The neurological examination and electrodiagnostic testing reveal evidence of left radial nerve damage. The sural autograft was utilized for repair, followed by the application of 1 mL of mesenchymal stem cell-derived exosome, comprising 5 billion microvesicles. This exosome was split into four equal volumes of 0.25 mL each and delivered microsurgically to both the proximal and distal stumps using the subepineural pathway. The patient was subjected to a period of 180 d during which they had neurological examination and electrodiagnostic testing.

RESULTS

The duration of the patient's follow-up period was 180 d. An increasing Tinel's sign and sensory-motor recovery were detected even at the 10th wk following nerve grafting. Upon the conclusion of the 6-mo post-treatment period, an evaluation was conducted to measure the extent of improvement in motor and sensory functions of the nerve. This assessment was based on the British Medical Research Council scale and the Mackinnon-Dellon scale. The results indicated that the level of improvement in motor function was classified as M5, denoting an excellent outcome. Additionally, the level of improvement in sensory function was classified as S3+, indicating a good outcome. It is noteworthy that these assessments were conducted in the absence of physical therapy. At the 10th wk post-injury, despite the persistence of substantial axonal damage, the nerve exhibited indications of nerve re-innervation as evidenced by control electromyography (EMG). In contrast to the preceding. EMG analysis revealed a significant electrophysiological enhancement in the EMG conducted at the 6th-mo follow-up, indicating ongoing regeneration.

CONCLUSION

Enhanced comprehension of the neurobiological ramifications associated with peripheral nerve damage, as well as the experimental and therapy approaches delineated in this investigation, holds the potential to catalyze future clinical progress.

Key Words: Mesenchymal stem cell; Exosomes; Radial nerve; Sural nerve

Core Tip: Peripheral nerve damage can manifest in several contexts, including civil, military, or iatrogenic circumstances. Despite the advancements in microsurgical techniques in recent times, the treatment outcomes for peripheral nerve damage have not yet reached a desirable level. This study investigates the functional recovery of a patient who received a sural nerve transplant and exosome application to treat a whole radial nerve lesion caused by a knife assault. Stem cell-derived treatments, such as the use of exosomes, have the potential to provide a novel and promising outlook for the treatment of peripheral nerve injury.

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INTRODUCTION

Peripheral nerve injury (PNI) can manifest in several contexts, including both civilian and military settings, as well as iatrogenic harm resulting from surgical interventions. The hand plays a crucial role in several everyday tasks, hence any impairment in its functionality might lead to significant challenges in one's daily life. Inadequate treatment modalities often lead to functional limitations, hence exerting adverse consequences on both familial units and broader societal structures. Sensory and motor dysfunction might potentially result in the full paralysis of a limb or the onset of unmanageable neuropathic pain.

According to a study, a significant proportion of nerve injury, namely up to 73.5%, is attributed to the upper extremities[1]. The radial nerve, an important peripheral nerve of the upper limb, plays a critical role in the motor function of the forearm, wrist, and fingers. The radial nerve is commonly categorized into four sections when discussing injuries: Infraclavicular, humeral shaft, from the lateral arm to the antebrachial fossa, and posterior interosseous nerve[2]. It was determined that, after an average follow-up duration of 21.5 mo, the results for injuries treated within five months of occurrence were more favorable in the distal subgroup compared to the proximal segment of the nerve. Furthermore, it has been asserted by Roganovic and Petkovic[3] that proximal radial nerve injuries provide more unfavorable results compared to intermediate and distal lesions.

The primary aim of nerve repair is to achieve reinnervation of the target organs through the guidance of regenerated sensory, motor, and autonomic axons, while minimizing the loss of fibers at the suture line. Currently, the ideal treatment strategy involves the utilization of tensionless epineurial sutures for end-to-end microsurgical repair. Autologous nerve transplantation is the recommended approach for reconstruction in cases when a nerve gap exists and direct end-to-end suturing is not feasible. Despite the presence of notable limitations, such as the morbidity associated with donor site and the limited length of graft material, other approaches including the use of natural or artificial conduits for tubulization operations are viable options for addressing small nerve deficits. Nevertheless, it is worth noting that nerve autografting remains the prevailing and most esteemed method for bridging nerve gaps at present.

The utilization of intraoperative nerve stimulation, the enhancement of motor nerve recovery, and the successful attainment of nerve exposure and mobilization are all advantageous elements of acute repair, often performed within a three-day timeframe. According to existing literature in the field of biochemistry, it has been documented that within a time frame of 72 h following an injury, nerve endings retain the presence of neurotransmitters[4]. From a histopathological standpoint, it can be observed that nerve endings, upon rapid transection, initially exhibit symmetrically aligned bundles of nerve fibers. However, as time progresses, the task of aligning these nerve ends becomes progressively more difficult due to the occurrence of Schwann cell (SC) proliferation, fibrosis, and angiogenesis at each respective end. One significant limitation associated with early nerve healing is the inability to accurately ascertain the specific site and extent of the lesion. The process of restoring nerve function following extended periods of time is sometimes referred to as delayed repair.

In their study, Shergill *et al*[5] observed that the outcomes were least favorable, with an overall failure rate of 42%, when dealing with uneven wounds and significant gaps in a cohort of 220 radial nerve gaps that underwent sural nerve grafting. Terzis and Konofaos[6] conducted a study in which they found that younger patients, those with denervation duration of three months or less, lesions in continuity, no accompanying nerve injuries, distal lesions, neurolysis, and nerve grafts of five centimeters or less in length, had improved functional results.

The presence of SCs represents a significant benefit in the context of autograft procedures. The generation of an optimal environment for axonal development is facilitated by the presence of live SCs and trophic substances within the graft. The aforementioned components, including SC basal laminae, neurotrophic factors, and adhesion molecules, together form a crucial scaffold. One advantage of an autogenous nerve transplant is its ability to avoid any immunoreaction. This is due to the graft's absorbable and permeable nature, allowing it to directly interact with its surrounding environment. Additional options for treatment involve the utilization of synthetic nerve guiding conduits. However, due to their deficiency in biological and cellular assistance, it is more advantageous to prioritize the preservation of a working nerve. One potential strategy to overcome these limitations is to introduce SCs or exosomes into the conduits, since they have shown the ability to facilitate axon regeneration[7]. Although the use of tissue engineering techniques to generate artificial conduits has been demonstrated to be advantageous for PNI, the results are still far from ideal. Numerous natural (such as vein grafts) and synthetic (artificial) materials have been subjected to testing in both clinical and experimental settings[7].

In contrast to autografts, nerve allografts do not need a further incision and have the advantage of an unrestricted supply of nerve tissue for transplantation. Moreover, the injured nerve of the receiver might potentially be substituted by a nerve of the same kind obtained from the donor. An enhanced motor recovery can be achieved by replacing a mixed sensory-motor ulnar nerve with a comparable mixed-type ulnar nerve procured from a donor, as opposed to utilizing a sensory-only sural nerve transplant. Although allogenic nerve grafts possess a restricted ability to provoke an immune response, the utilization of immunosuppressive medication is necessary to avert graft rejection. In contrast to the central nervous system (CNS), the peripheral nervous system (PNS) has the capacity for regeneration following injury. In the PNS, SCs are responsible for the release of growth factors and the removal of debris.

The activation of macrophages and subsequent formation of a new medullary sheath is initiated by the presence of myelin and axonal debris. Nevertheless, achieving good outcomes poses a challenge due to factors such as sluggish neuron regeneration, Wallerian degeneration, tissue adhesion, and muscle atrophy. PNI results in the occurrence of Wallerian degeneration, a process characterized by the infiltration of macrophages into the damaged nerve on the third day post-trauma. These macrophages secrete substantial quantities of variables, including C-C motif ligand 2, tumor necrosis factor-α, interleukin (IL)-1α, and IL-1β[8].

The growth rate of regenerating axons is often limited to around 1 millimeter every day. The process of regeneration is facilitated by various mechanisms, which encompass mechanical components like Büngner's cell bands, pathway-markers that are localized at the axons and SCs, chemical factors such as cytokines that have a more localized action, and growth factors like ciliary neurotrophic factor, epidermal growth factor, platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, and nerve growth factor (NGF) that have a more distant effect.

Autologous nerve transplantation remains the established benchmark in the treatment of peripheral nerve abnormalities; nonetheless, it is imperative to explore other approaches. The local administration of stem cells or exosomes has been shown to have the potential to augment axonal regeneration and promote the creation of myelin sheaths in the treatment of PNI. Several factors, such as fibroblast growth factor, NGF, ciliary neurotrophic factor, brain

derived neurotrophic factor, and glial cell line-derived neurotropic factor, have been identified as potentially advantageous for promoting the survival of neural cells and facilitating nerve regeneration. These factors are released by stem cells during the process of tissue repair[9]. While stem cell-based therapies have shown beneficial effects on tissue regeneration, it has been noted that the fundamental mechanism responsible for stem cell-mediated tissue healing is paracrine signaling rather than stem cell differentiation[10]. There exists a considerable amount of empirical data indicating that exosomes, with a notable capacity to serve as an innovative alternative to whole cell treatment, are capable of facilitating the paracrine activity of stem cells[11]. Moreover, it has been shown that the utilization of exosomes is comparatively safer in comparison to stem cell therapy. The administration of some interventions has the potential to overcome cellular immune rejection and carcinogenic mutations[12].

In a recent study, it was shown that SCs have the ability to produce exosomes that can promote the regeneration of axons. This effect was observed both in laboratory settings (*in vitro*) and in living organisms (*in vivo*)[13]. The internalization of SC exosomes by peripheral nerve axons suggests a probable specificity of their payload in relation to the development, protection, or regeneration of the PNS. According to a study conducted by Kingham *et al*[14], it was shown that adipose-derived stem cells produce exosomes that have resemblance to SCs. These exosomes contain identical cargo and have the ability to promote the rebuilding of axons.

Mesenchymal stem cells (MSCs) are a type of stem cell that possess multipotent capabilities and are obtained from various mesenchymal tissues such as bone marrow, adipose tissue, dental pulp, umbilical cord blood, and others. Previous studies have demonstrated that multipotent MSCs have the potential to significantly improve functional recovery following nerve damage[15]. A recent study has provided evidence that MSCs release exosomes, which play a significant role in intercellular communication and the maintenance of dynamic and balanced microenvironments necessary for tissue repair[16]. Exosomes, which measure between 40 and 100 nm, are the most diminutive membranous vesicles. Different types of cells, including neurons, tumor cells, and kidney cells, secrete nanovesicles. These nanovesicles may be detected in a range of bodily fluids, such as urine, amniotic fluid, malignant ascites, bronchoalveolar lavage fluid, synovial fluid, breast milk, saliva, blood, and cerebrospinal fluid. Exosomes exhibit variations in their protein, lipid, noncoding RNA, mRNA, and microRNA (miRNA) composition, together referred to as "cargo" contents, depending on their parental origin. These cargo contents are then transported to adjacent cells or sent to cells located at a distance. Remarkably, recent research has shown that a multitude of cells inside the nervous system have the ability to produce exosomes, which are extracellular membrane vesicles. This observation indicates their active participation in the operation, growth, and disorders of this particular system. Recent studies have provided evidence for the importance of miRNAs in exosomes as mediators of intracellular communication between donor and recipient cells[17]. The ability of these entities to traverse the blood-brain barrier has several prospects in the field of neuroprotection. This phenomenon is evidenced by their active participation in the process of neuronal repair and the restoration of peripheral nerves[18].

Exosomes generated by MSCs have the capacity to activate phosphatidylinositol 3-kinase/protein kinase B, extracellular signal-regulated kinase, and signal transducer and activator of transcription 3 signaling pathways to promote the expressions of growth factors such as insulin-like growth factor-1, NGF, and stromal-derived growth factor-1[19]. Exosomal miRNAs (including miR-199b, miR-218, miR-148a, miR-135b, and miR-221) obtained from MSC culture have been shown to affect neuron differentiation, proliferation, vascular regeneration, and axonal outgrowth in several studies [20]. Exosomes generated by MSCs from different sources have been shown in various studies to stimulate nerve regeneration; this effect is probably related to exosomal miRNA[21]. The function of exosomes produced by MSCs depends on the state of the origin cell, which may affect the miRNA content of exosomes and thus influence their biological function[22]. Additionally, altering MSCs to overexpress miRNAs can result in exosomes that are miRNA-enriched and could be a useful strategy to accelerate peripheral nerve regeneration[23]. The process of exosome formation and their cellular functions are notably modulated by proteins present within exosomes. Exosomes have been identified as carriers of actin and β-tubulin, two essential membrane and cytoskeletal proteins involved in the process of axonal growth.

Previous studies have demonstrated the indispensability of heat shock protein 70 in providing metabolic support and safeguarding neurons[24]. The protein galectin-3, which is associated with the phagocytosis of myelin, has been detected in exosomes and has been shown to be increased by SCs following nerve injury[13]. In their study, Krämer-Albers *et al*[25] documented the presence of myelin proteins, such as myelin-associated glycoprotein and proteolipid protein, within exosomes. The authors emphasized the significant contribution of these proteins in the process of nerve remyelination.

The denervation of SCs leads to alterations in the synthesis of several substances, resulting in both an increase and reduction in their production. This procedure facilitates the shift of SCs from a phenotype characterized by proliferation and myelinization to a regenerative phenotype within the initial 24-h period. A delicate equilibrium exists between degenerative and regenerative mechanisms. The presence of exosomes harboring diverse compounds is likely to expedite the process of regeneration.

According to research findings, exosomes have been observed to possess significant amounts of IL-6, IL-8, and several other cytokines[10]. The aforementioned results together indicate that exosomes include a diverse array of components that play a vital role in the regeneration and remodeling of the nervous system. Exosomes are also shown to possess DNA, however its specific function is yet to be determined[26]. Exosomes participate in cellular communication, contribute to the presentation of antigens by immune cells, and exhibit either pro-inflammatory or anti-inflammatory properties[27].

Maintaining vascular integrity is crucial for the maintenance of the milieu of the nervous system, ensuring its homeostasis and facilitating the processes of nerve system healing, development, and optimal functioning. The use of exosomes originating from MSCs has been shown to have a positive impact on the restoration of function following nerve injury in rats. This is achieved through the activation of the body's own processes of angiogenesis and neurogenesis, as supported by previous research[28]. Additionally, these entities may possess clinical therapeutic potential and function as

paracrine agents that stimulate the growth of new blood vessels[29]. This study proposes that exosome-mediated intercellular communication within the nervous system facilitates the activation of angiogenesis by neurons and MSCs, therefore establishing exosomes as a crucial instrument in the process of peripheral nerve regeneration.

The complexity of cellular and molecular processes involved in peripheral nerve regeneration has become apparent, indicating that microsurgery alone is insufficient for effective nerve repair. There are several reasons that contribute to the unfavorable results observed in nerve repair. These include the sluggish, inadequate, and misdirected regeneration of axons, the atrophy of end-organs and the failure of reinnervation, as well as the fast and long-lasting reconfiguration of the cortical regions involved[30].

Despite the accumulation of substantial information regarding neuropathophysiology throughout the last three decades, the fundamental principles governing clinical interventions for nerve damage have remained unaltered. Consequently, the clinical results associated with such interventions have failed to meet expectations. Potential future techniques to the repair of peripheral nerves include cell-based supportive treatment and the bioengineering of nerve conduits. Current research highlights the increasing importance of cell-based treatments that provide assistance in the process of nerve regeneration[31]. The initial pilot investigation presented in this paper describes the treatment of a clinical case using sural autograft and perioperative implantation of exosomes produced from Wharton's jelly-derived MSCs (WJ-MSCs).

MATERIALS AND METHODS

Ethics and consent

The current investigation received approval from the medical ethics committee of the authors' institution, with the assigned protocol number 56733164-203-E.5863. Upon entering the trial, the patient provided written informed permission. patient provided informed consent for their use in the study. The patient's explicit endorsement, as demonstrated by their written informed permission, established their willingness to participate in future clinical investigations.

Isolation of the exosomes from WJ-MSCs

Various approaches have been employed for the extraction of exosomes, including differential ultracentrifugation, density gradient separation, ultrafiltration, size exclusion chromatography, immunoisolation, and flow cytometry. Differential ultracentrifugation is now the prevailing technique employed for exosome purification, since it has been widely acknowledged as the benchmark approach for separating exosome subpopulations characterized by generally consistent sizes (Supplementary Table 1, Supplementary Figures 1 and 2).

The WJ-MSC cells were cultivated until they reached 90% confluency. Subsequently, the cell medium was replaced with serum-free MSC NutriStem® XF Medium, and the cells were cultured in a humidified atmosphere for a minimum of 48 h. After the incubation period, the medium was collected and subjected to centrifugation at 300 × *g* for 5 min, followed by centrifugation at 1000 × *g* for 10 min in order to eliminate the cells and cellular debris, respectively. Subsequently, a centrifugation phase was conducted at a force of 5000 times the acceleration due to gravity (5000 × *g*) for a duration of 20 min in order to eliminate nuclei and cellular debris. Subsequently, the clarified supernatant underwent ultracentrifugation at a force of 100000 times the acceleration due to gravity for a duration of 70 min in order to achieve the concentration of exosomes (OPTIMA MAX-XP ultracentrifuge, Beckman Coulter, United States). The protein content of 100 mL vesicles was determined using the BCA protein assay method to confirm the production of MSC-derived exosomes. The BCA protein Assay Kit method was used in order to show the presence of protein in the exosomes. After the exosomes had been isolated, 20 µL of the resulting pellet was prepared by adding 200 µL of working solution (50:1 Reagent A:B) following the kit protocol and incubated for one hour at 37 °C. After incubation, the amount of protein was determined by spectrophotometer at 562 nm according to the BSA standard (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, United States). The pellet was resuspended in 500 µL of Dulbecco's phosphate buffered saline, with a pH of 7.4, and stored at a temperature of -80 °C until it was utilized. To examine the characteristics of exosomes, the researchers employed a method where the isolated exosomes were tagged with well-established tetraspanin markers (CD81; 97%, CD9; 79%, and CD63; 95%) (Supplementary Figure 3). Subsequently, flow cytometry was utilized to study these labeled exosomes (BD Facs Canto, United States). In addition, the morphology and size of isolated exosomes were evaluated *via* transmission electron microscopy. Dynamic light scattering was also used to determine the size distribution of MSC-derived exosomes.

Surgical procedure and exosomes implantation

An immigrant man of 24 years' age, who was right-hand dominant, appeared with a stab wound to his left arm below the elbow. There was a strong indication of radial nerve palsy since the patient could barely extend their wrist, fingers, or thumb upon first presentation. This is because the nerve branch that supplies the extensor carpi radialis longus muscle was not severed. The extensor carpi radialis brevis (ECRB) muscle was assumed to be entirely impaired, along with the other extensors of the fingers (Video). After being sent to the cardiovascular surgery unit from the emergency room on the off chance that the patient had sustained a vascular damage, it was determined that no such injury had occurred. The patient's muscular strength in the neurological examination was measured at 1/5 in wrist and finger extension and 1/5 in forearm supination on the patient's left side. The dorsal surface of the left hand save for the dorsal side of the little finger was in anesthetic state. After evaluating the patient's nervous system, it was decided to contemplate amputating the radial nerve completely below the elbow (before the superficial and deep branches). Electrodiagnostic tests performed

before to surgery (on the third day after the accident) revealed a lack of motor response in the left radial nerve segment below the elbow.

Subsequently, a surgical procedure to investigate the radial nerve in the left forearm and the subsequent implantation of exosomes were scheduled for the patient. The patient was placed in a supine posture with the forearm extended on a hand table while under general anesthesia. Additionally, the surgical incision site was appropriately demarcated. In cases where primary nerve repair is not feasible, the localization of the left sural nerve was duly noted and prepared under sterile conditions. This was done to facilitate the potential acquisition of a sural autograft, if required, and to enable subsequent nerve repair utilizing the autograft. A curvilinear incision was made, starting at the lateral antecubital fossa and extending down the medial side of the brachioradialis muscle to the midway of the forearm, including the area associated with stabbing. The brachioradialis muscle was detected and subsequently retracted in a lateral direction. Following this, the superficial branch of the radial nerve and the radial arteries were located underneath it. The radial blood arteries exhibited no signs of damage or disruption. In order to locate the primary branch of the radial nerve, the brachioradialis muscle was laterally retracted, and the radial superficial nerve was traced proximally until the radial nerve was successfully located. The radial nerve was dissected in a distal manner, leading to the identification of the branches associated with the posterior interosseous nerve and ECRB. A full avulsed injury (neurotemesis, Sunderland categories grade 5) was detected in the nerve just prior to the branching of the posterior interosseous nerve and ECRB. Once the nerve had been resected to restore its healthy proximal and distal extremities, the resulting segments were joined and the distance between them was determined. Approximately 7 centimeters of space was present, and an 8.5 centimeter sural autograft was obtained. Approximately sixty percent of the cross-sectional area of the damaged nerve was covered by the autograft. Grafting was accomplished using 8-0 prolene to create four epineural sutures on each side while observing through an operative microscope at a magnification of × 12. Following that, a subepineural route was utilized to microsurgically apply 0.25 mL of 1 mL of exosome derived from MSCs (containing 5 billion microvesicles) to both sides of the proximal and distal stumps (Figure 1). Each portion contained 5 billion microvesicles. A photograph taken intraoperatively is illustrated in Figure 2. To mitigate the unintended dissemination of the exosome during injection into the adjacent tissue, fibrin adhesive was administered to the affected areas. In order to facilitate neovascularization in the adjacent tissues, a minute quantity of exosomes administered *via* ubepineural route was permitted to traverse into the surrounding tissue. Technical difficulties prevented intraoperative neurophysiological monitoring from being conducted throughout the operation; therefore, tourniquets were not utilized. Two weeks after the operation, the hand was immobilized in the most functionally advantageous position conceivable. With early motor reeducation, we intended to initiate intensive and protracted physical therapy of the hand two weeks postoperatively; at one month, we initiated light strengthening and moderate range of motion exercises to alleviate edema. Opportunistic behavior on the part of the patient rendered the physical therapy and rehabilitation program unfeasible to incorporate. Ten weeks subsequent to the injury, he demonstrated active wrist extension with a muscle strength rating of 3/5 (Video). Electrodiagnostic testing revealed that although the extension of the finger and hand had commenced, rehabilitation and reinnervation were still in the early stages, characterized by severe axonal injury. During sensory evaluation, it was noted that the senses of contact and pain had been restored without any 14-excessive reactions. During the control examination conducted six months postoperatively, the strength of the muscles involved in extending the wrist, fingers, and thumb was assessed to be +4-5/5 (Video). Additionally, the patient's sensory examination exhibited a near-complete improvement.

RESULTS

Assessment of motor and sensory functions

The efficacy evaluation encompassed assessments of both motor and sensory functions. An assessment of the motor and sensory nerves' recovery was conducted in adherence to global benchmarks. Strength and range of motion assessments are components of the motor function evaluation. In order to evaluate sensory functions, static and dynamic two-point discrimination was conducted. Pain, the patient's or physician's assessment of the improvement in function, and electromyography (EMG) were additional examinations. Each assessment was conducted bilaterally on the upper limb.

Motor and sensory functions were assessed using the Mackinnon-Dellon scale (Table 1) and the British Medical Research Council (BRMC) scale (Table 2), the two most widely used methodologies for evaluating outcomes following repair of PNI, respectively[32]. The improvement in sensitivity was evaluated using a four-point scale, whereas the enhancement in motor functions was assessed using a five-point scale. M0-M2 and S0-S1 outcomes were considered to be inadequate. Improvements of M4 and S3 or greater were deemed "excellent" and "very good", respectively. In conclusion, both M3 and S2 were classified as "good". This study utilized the most commonly applied criteria: adequate motor recovery (grade M4 or M5), and satisfactory sensory recovery (grade S3+ or S4).

The duration of the patient's follow-up was 180 d. An improvement in Tinel's sign and sensory-motor recovery was observed as early as the tenth week following nerve transplantation. Upon the conclusion of the 6-mo follow-up phase, the nerve's motor and sensory functions (as measured by the Mackinnon-Dellon scale and the BRMC scale) returned to M5 (outstanding) and S3+ (good), respectively, without physical therapy.

Electrophysiological assessment

Electrophysiology was utilized in order to gauge the electrical conduction of the nerve. The thickness of the myelin sheath and the quantity of myelinated nerve fibers are both factors that influence electrical conduction. EMG exhibits greater accuracy in detecting early re-innervation compared to physical examination. Consequently, upon needle examination of the muscle closest to the site of injury, the recovery of motor unit action potentials is frequently the initial indication of re-

Table 1 Classification of sensory recovery according to the Mackinnon-Dellon scale

Grade	Recovery of sensibility
S0	Poor: No recovery of sensibility
S1	Poor: Recovery of deep cutaneous pain sensibility
$S1^+$	Poor: Recovery of superficial pain sensibility
S2	Poor: Recovery of superficial pain and some touch sensibility
$S2^+$	Poor: As in S2, but with overresponse
S3	Poor: Recovery of pain and touch sense with no overresponse (> 15 mm s2PD, > 7 mm m2PD)
$S3^+$	Good: As S3, but localization of stimulus is good and imperfect recovery of 2PD (7-15 mm s2PD, 4-7 mm m2PD)
S4	Excellent: Complete recovery (2-6 mm s2PD, 2-3 mm m2PD)

The Mackinnon-Dellon scale is a tool used to assess sensory function after the repair of peripheral nerve injuries. This scale is used to evaluate the degree of sensory recovery in patients who have undergone nerve repair surgery. The scale ranges from S0 to S4, with S0 indicating no sensation and S4 indicating normal sensation. s2PD: Static sense of two-point discrimination; m2PD: Motor sense of two-point discrimination.

Table 2 The British Medical Research Council scale

Grade	Recovery level	Muscle strength
M0	Failure	No contraction
M1	Poor	Return of perceptible contraction in the proximal muscle group
M2	Fair	Return of perceptible contraction in both proximal and distal muscles; the extensor carpi radialis muscles may contract against force, but absence or trace of wrist extension
M3	Moderate	Wrist extension against gravity to the neutral position; absence or trace of finger or thumb extension
M4	Good	Wrist extension against force; trace or better finger and thumb extension
M5	Excellent	Wrist, finger, and thumb extensors restored close to normal

The British Medical Research Council scale is a tool used to assess muscle power in patients with peripheral nerve injuries. The scale grades muscle power on a scale of M0 to M5 in relation to the maximum expected for that muscle. Motor recovery grading following the recovery of radial nerve for a proximal forearm radial nerve lesion, extensor carpi radialis longus and brevis are the proximal muscles, and the extensor communis and extensor pollicis longus are the distal muscles.

innervation. Neurophysiological indicators of axonal regeneration often manifest weeks to months following PNI, prior to the manifestation of voluntary contraction. Preoperative electrodiagnostic testing conducted on the third day following the injury revealed the absence of any motor response (total denervation) in the left radial nerve segment below the elbow. The patient demonstrated significant improvement as indicated by BMRC scores prior to the scheduled date of the control EMG. Notwithstanding the persistent extensive axonal damage, indications of nerve re-innervation were detected on the control EMG of the nerve (at the tenth week after the injury). Physical therapy and rehabilitation were additional treatment modalities that the patient was incompatible with. In contrast, the sixth-month control EMG revealed a notable electrophysiological improvement in comparison to the previous EMG; furthermore, the regeneration process persisted.

DISCUSSION

Despite advancements in microsurgical techniques and comprehension of the pathophysiology underlying PNS injury and regeneration, PNIs continue to pose a substantial obstacle. The PNS possesses the ability to restore and regenerate by nature. PNIs elicit a substantial cellular and molecular reaction that involves not only the damaged neurons but also the supporting SCs. Antidromic electrical activity, which initiates kinase cascades and activates calcium channels, is the initial signal received by the neuronal cell body following axonal injury. This results in a substantial response in both protein and gene expression; the equilibrium of protein and gene expression determines whether the neuron survives and attempts to regenerate or undergoes apoptotic death.

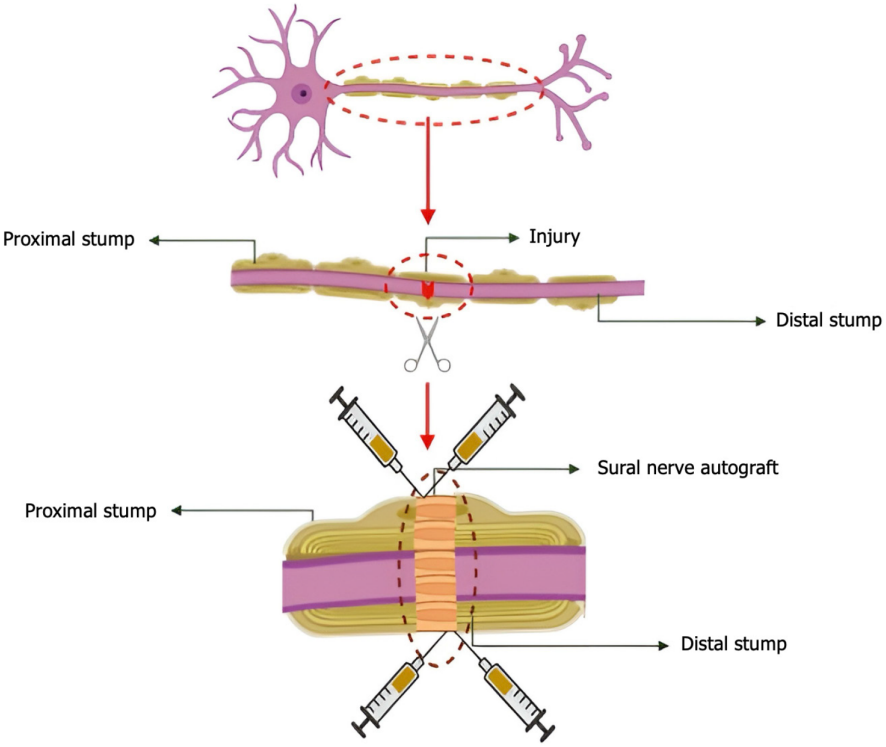

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Figure 1 Illustration of application. Sural nerve grafting was performed using 8-0 prolene to create four epineural sutures on each side. The subepineural route was used to microsurgically apply 0.25 mL of 1 mL exosome (containing 5 billion microparticles) to both sides of the proximal and distal stumps.

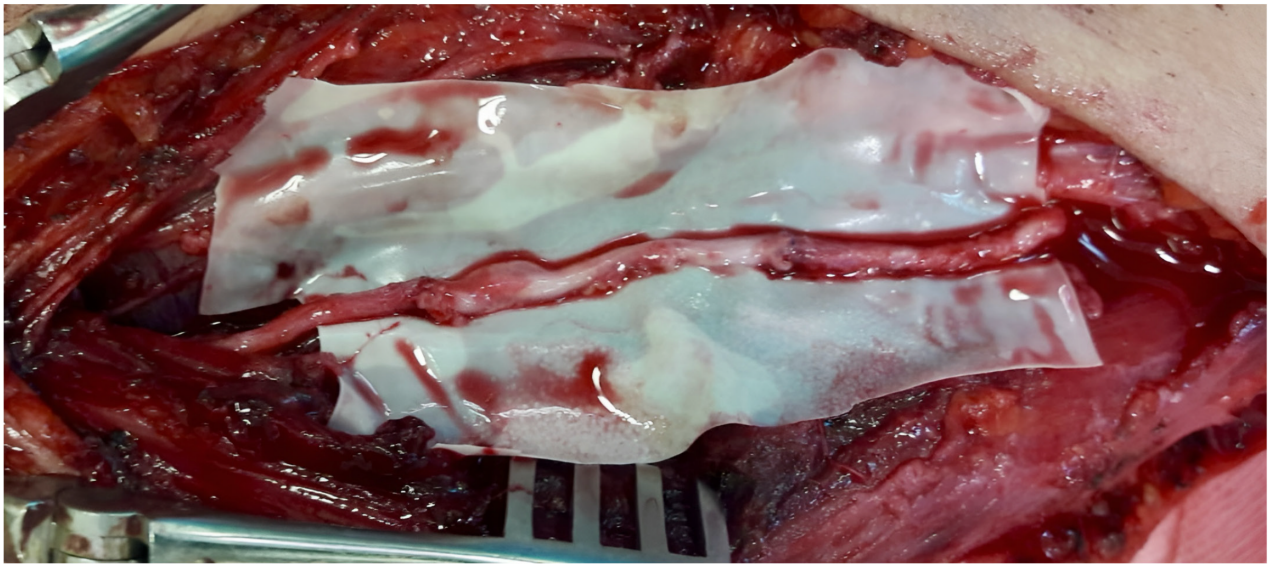

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Figure 2 An intraoperative photograph. A gap of approximately 7 cm was observed in the injured radial nerve trace. Sural nerve graft was taken and repair of this gap was achieved with microsurgical technique. In the photograph, the light coloured part in the middle along the nerve line shows the sural nerve graft.

The degeneration of the axon and myelin in the distal stump occurs within a very short timeframe, typically within a few hours. Subsequently, macrophages gather at the site of injury, playing a crucial role in the clearance of cellular debris. During the initial 24-h period, SCs undergo proliferation and transition from a myelinating state to a regenerative one. This transformation is accompanied by an increase in the expression of several molecules that play a role in both the degenerative and regenerative processes occurring simultaneously[33]. Following the clearance of debris by SCs and macrophages, SCs initiate the formation of Büngner bands, which create a trophic-rich environment that facilitates directed axonal regeneration. Similarly, the target organ that has been denervated experiences a depletion of trophic factors, resulting in the atrophy of muscle fibers and the death of satellite cells.

The regenerative capacity is contingent upon several factors, including the patient's age, the specific type of damage, and notably, the proximity of the injury to the nerve cell soma. Regeneration following nerve damage involves several crucial factors from a pathophysiological perspective, including the activities of macrophages and SCs, the inflammatory response, and vascular regeneration. The degree of patient participation during the therapy process has a notable influence on the probability of obtaining recovery, particularly among patients with a moderate to high socio-cultural status who tend to exhibit the most favorable outcomes. In contrast to analogous lesions observed in older individuals or those with a generally compromised state of health, adolescents and teens consistently exhibit a more favorable trajectory and result. Matejcík[34] conducted a study which revealed that those under the age of 20 had the most favorable results. In contrast to injuries of a more complex nature, such as lacerations and contusions, pure severance injuries have been found to provide more favorable circumstances for effective autotransplantation. According to Matejcík[34], it was shown that injuries in close proximity to a certain anatomical region had the most unfavorable results in relation to the severity of the damage. The most favorable results were observed in injuries located further out from the center, specifically around the wrist, with a success rate of 87.6%[34].

Following an injury, nerves undergo a process of progressive regeneration, wherein they must successfully extend, identify, and reestablish connections with the anatomical structures under their control. In order to prevent degeneration, it is imperative that endoneurial tubes establish contact with regenerated axons within a timeframe ranging from 18 to 24 mo subsequent to the damage. Following a period of denervation lasting between 12 and 18 mo, the atrophy of the target muscle reaches a point where it becomes permanent, hence imposing limitations on the functional efficacy of the healing process. Although sensory function has the potential to be restored at a later stage, even several years following the initial injury, it is worth noting that sensory receptors persist for a significantly extended duration.

Nerve damage of varied degrees can be a consequence of an injury, necessitating the potential requirement for reconstructive surgery. The primary objectives of this surgical procedure are to provide optimal enhancements in both motor and sensory functions within the denervated region located distally. However, despite the use of meticulous surgical techniques and a range of corrective interventions, achieving a complete restoration of functionality, especially in terms of motor function, is seldom attainable. Primary nerve repair is considered the most effective approach for restoring functionality in instances of acute nerve transections characterized by abrupt injuries, minimal or absent compression, sufficient blood circulation, and uncontaminated wounds. In order to optimize nerve regeneration following the healing process, it is important to ensure that nerve stumps are aligned in a precise manner, devoid of any strain. Moreover, the restoration procedure should be conducted atraumatically, minimizing tissue damage and the number of sutures employed. Autologous nerve grafting is now considered the most effective method for repairing nerve gaps that cannot be brought together or joined without strain. In contrast to direct repairs conducted under conditions of high strain leading to nerve ischemia, nerve grafts had much superior results.

Artificial nerve guidance scaffolds have been developed with the aim of facilitating nerve regeneration by the restriction of myofibroblast infiltration, reduction of scar formation, and the concentration of neurotrophic substances. The regeneration capabilities of commercially available technologies, often consisting of hollow tubes composed of biodegradable polymer or collagen, have not been able to attain the same levels as autologous nerve grafting. These technologies are limited to treating tiny lesions (less than 2 cm) and demonstrate inadequate functional recovery[35]. A novel methodology integrates stem cells into biomaterial scaffolds, therefore amalgamating neuroprotective interventions with nerve restoration and enhanced axonal regeneration. Successful regeneration has not been achieved only *via* the use of nerve guides, making it particularly crucial for addressing big gaps.

Several variables, such as surgical delay, patient age, injury type, autograft length, injury location, and nerve damage type, might potentially influence the success of peripheral nerve repair using autografts. The results of nerve injury repair in the upper limbs were shown to be more favorable compared to those in the lower limbs. The temporal interval between the occurrence of the damage and the subsequent reconstructive surgical intervention had a crucial role in achieving favorable outcomes, particularly among individuals in younger age groups.

The determination of the anatomic nerve with the most favorable prognosis recovery has been extensively studied, yielding a multitude of conflicting findings. Several studies have shown contrasting findings on the optimal healing of the median nerve and radial nerve in the upper extremities[36]. Furthermore, additional research conducted on the anatomical peripheral nerves pertaining to motor-sensory recovery failed to demonstrate any statistically significant disparities[37]. The occurrence of "crossing over" inside the regenerated nerve has been shown to result in a reduced likelihood of complete recovery for mixed nerves, such as the proximal section of the ulnar or median, compared to pure nerves, such as the motor branch of the ulnar or median[36].

The study conducted by Renner *et al*[38] shown a significant improvement in motor function for around 75% of patients with radial nerve injuries who underwent nerve grafting. Nevertheless, the present study lacks data regarding the administration of postoperative physical therapy to the patients as well as the duration required for complete recuperation. A suggested minimum follow-up time of one to two years is typically advised for the repair of the median and ulnar nerves. Additionally, the final functional evaluation should be conducted two to three years after the repair in children and adolescents, and five years after the repair in adults[39]. The existing research does not provide any information on the optimal period for evaluating outcomes in radial nerve repair. Furthermore, the existing research does not provide conclusive evidence about the specific timeframe for the onset of electrophysiological or clinical improvement following primary repair or autograft repair.

In the present case study, it is noteworthy that the patient did not undergo post-operative physical therapy. However, it is important to highlight that both clinical and electrophysiological recovery started at the 10th wk, with nearly full restoration of motor and sensory functions documented during the 6th mo follow-up assessment. In the present study, the duration of follow-up was limited to a maximum of six months subsequent to the surgical procedure and administration of exosomes. This constraint was imposed due to the patient's non-adherence to the prescribed treatment regimen, hence

preventing a more extensive follow-up period that would have encompassed physical therapy and rehabilitation interventions.

The proximal part of the nerve tract is often composed of mixed nerve bundles. As a result, there exists significant potential for interplay and development between sensory and motor nerve fibers. The technique of perineural suturing involves the joining of the motor and sensory tracts' respective ends, hence promoting a favorable functional recovery. This approach is effective due to the preexisting separation of the nerve into distinct sensory and motor tracts at the distal end. Consequently, the process of regeneration is prolonged in injuries that occur closer to the point of origin.

The sural nerve is commonly used as the donor nerve. When the sural nerve proper is harvested alone, a graft material exceeding 20 cm can be obtained. Conversely, when it is harvested in conjunction with the medial sural cutaneous nerve, a maximum of 50 cm can be achieved. According to a study, it was shown that sural nerve autografts had the most unfavorable motor and sensory results[37]. Furthermore, it has been established that there is a negative correlation between graft length and clinical outcomes. Matejcík[34] found that the duration of the transplant procedure exerts a detrimental influence on the overall success rate of transplantation. According to the study conducted by[34], the success rate of grafts measuring up to 5.0 cm was found to be 80.6%. However, when the length of the grafts surpassed 10 cm, the success rate dropped significantly to 16.7%. In the presented case study, it was necessary to utilize a sural autograft of considerable length (8.5 cm) in response to the significant distance separating the nerve ends. Nevertheless, a rapid and nearly full clinical recovery was attained.

The temporal interval between the occurrence of the injury and the subsequent undertaking of reconstructive surgery plays a pivotal role in influencing the results of surgical interventions aimed at restoring the functionality of peripheral nerves. The influence of this ingredient is more pronounced in younger patients[34]. The surgical procedure for the patient was scheduled to take place on the third day following the occurrence of the injury, aligning with the anticipated arrival time of the exosome sourced from the laboratory accredited under Good Manufacturing Practice standards.

The two primary factors that significantly impact axonal outgrowth *via* the transplanted nerve are the diameter of the grafted nerve fragment and the vascularity of the surrounding tissue bed. The process of nerve revascularization in nonvascularized autografts relies on the crucial mechanism of diffusion from the surrounding tissues. The clinical observations indicate that grafts with smaller calibers have more favorable outcomes. Nerve grafting entails the manipulation of regenerating fibers to traverse two coaptation sites, hence heightening the risk of axonal loss due to the development of scar tissue and the potential diversion of fibers into the perifascicular and epineurial connective tissue at each suture line. Similarly, a tissue bed that lacks sufficient vascularization hinders functional results, retards nerve regeneration, and enhances scar formation.

Inflammation has a big effect on peripheral nerve regrowth, and more and more research shows that cytokines and inflammatory reactions are key factors in this process[40]. Inflammation is needed to get rid of waste so that nerves can grow again, but it can also lead to problems like neuropathic pain and slow down nerve growth. So, the right amount of inflammation is important for nerves to heal properly. Exosomes from MSCs are known to help new blood vessels grow in nearby tissues[41]. Along with their ability to change the immune system, exosomes may also stop scars from forming in and around the restored peripheral nerve and at two places where they connect. The application of subepineural exosomes after surgery to repair a peripheral nerve may have sped up nerve healing by controlling the growth of new blood vessels in the nearby tissue.

Cell-to-cell contact is very important for maintaining balance in the body, especially in the nervous system. New research shows that exosomes can carry information between cells, which is important for the health and growth of brain systems[42]. Exosomes can help cells talk to each other in a number of different ways. One way is that miRNAs are sent from exosomes to target cells. These miRNAs can control how genes are expressed and how signals are sent in the receiver cells. Exosomes can also move proteins and lipids to other cells, which can change how cells work by changing their roles like growth, development, and death. Exosomes can also work with immune cells and parts of the extracellular matrix to control inflammation and metabolic balance[43]. These results make it seem like there are good ways to keep looking into new ways to help peripheral nerves grow back. When a peripheral nerve is damaged or dying, SCs around it move vesicles with polyribosomes into the axon, where the contents are released[44]. As a result, exosomes help get mRNA and ribosomes to damaged nerves, where they start the protein production that is needed for healing.

In the context of regenerative medicine for nerve repair, the utilization of cell-based treatments, namely stem cell therapy, holds significant promise in harnessing the regenerative capabilities of cells. Numerous *in vitro* and *in vivo* studies have been conducted to evaluate the potential of neural stem cells, SCs, olfactory ensheathing cells, induced pluripotent stem cells, and adult MSCs derived from different sources, for the purpose of nerve repair[45]. In relation to the origin of the cells, the potential for teratoma development, and the likelihood of unintended cellular differentiation, the utilization of MSCs in the field of regenerative medicine presents a reduced number of ethical concerns. These entities have the potential to facilitate the process of remyelination and provide trophic assistance to neurons undergoing regeneration.

Patient-specific stem cell exosomes might potentially be utilized as a strategy to enhance nerve regeneration. Nevertheless, in order to acquire stem cells, it is necessary to sacrifice a nerve that is in good health. The available evidence indicates that exosomes derived from matured MSCs that contain miRNAs have the potential to enhance axonal regeneration. Additionally, these exosomes may also indirectly facilitate the process of nerve repair by modulating the inflammatory response, hence promoting recovery[46].

Multiple studies have demonstrated the efficacy of MSCs in enhancing the process of peripheral nerve regeneration. Nevertheless, some notable limitations, including as immunogenicity, retention, and neoplasticity, have also been documented in the literature[46]. Exosomes, which are a form of acellular treatment, has a reduced immunogenicity that allows them to alleviate the limitations associated with MSC transplantation while maintaining their biological functionality. Hence, exosomes has the potential to be utilized in the development of groundbreaking therapeutic

interventions for the restoration and regeneration of peripheral nerves.

As previously indicated, optimal outcomes are achieved when regenerated axons are able to traverse a single coaptation site, hence facilitating tension-free end-to-end nerve repair. On the other hand, in the case of utilizing a nerve graft, the regenerating axons are required to traverse two healing sites, each potentially undergoing an independent inflammatory process that might result in further axonal degeneration. It is believed that the utilization of a subepineural exosome on the coaptation regions demonstrates efficacy in the treatment of the inflammatory process that arises inside these areas.

In the future, the combination of appropriate surgical intervention and postoperative rehabilitation programs has the potential to yield ideal outcomes in the treatment of peripheral nerve abnormalities. Considering the CNS's pivotal role in determining the functional result of peripheral nerve regeneration, it is imperative to prioritize the stimulation of cortical and subcortical remodeling as part of CNS-level rehabilitation. Hence, it is advisable to prescribe physical therapy and rehabilitation regimens for individuals having surgical procedures.

The primary constraint of our study resides in the utilization of a solitary example, which may engender erroneous deductions. Furthermore, the limited number of participants hindered the possibility of conducting a distinct examination of various nerve clusters. Hence, in order to evaluate our findings, it is imperative to conduct controlled, prospective, and randomized studies including bigger case series and other nerve groups.

Furthermore, the utilization of intraoperative electrophysiologic evaluation has gained recognition as an essential technique in the management of lesions in continuity. Assessing the whole amount of internal nerve damage only by macroscopic examination is a significant challenge, especially in cases of PNI accompanied by neuroma formation. The utilization of electrical stimulation for confirmation purposes plays a pivotal role in optimizing surgical efficiency. Electrophysiological monitoring was not conducted during the surgery. In the present study, the utilization of intraoperative EMG was deemed unnecessary due to the prompt surgical intervention conducted on the third day following the occurrence of the injury, hence resulting in a minimal likelihood of neuroma development. The utilization of intraoperative electrophysiological monitoring is recommended in surgical procedures to assess the functional integrity of vulnerable brain components.

In our perspective, the utilization of perioperative subepineural exosome application does not entail a significant expenditure of time. Despite the potential for increased expenses, this approach has the advantage of facilitating a prompt resumption of regular daily activities and professional obligations. Given the promising results observed thus far with autogenous nerve grafts, it is imperative to do a cost-benefit analysis of this novel approach. The findings of our study indicate that the utilization of exosomes formed from WJ-MSCs, in conjunction with microsurgical repair, holds significant potential as a new approach for nerve repair and regeneration. Additionally, our findings are anticipated to provide valuable insights for future investigations on the reparative methodologies for PNI.

The potential enhancements in nerve repair results by modifications to microsurgical techniques are unlikely to be substantial[47]. Therefore, it is imperative to conduct clinical trials to examine the therapeutic benefits of exosomes, since they have previously been explored in experimental studies. Currently, there is a lack of prospective randomized double-blind research pertaining to this particular issue in the existing literature. Hence, case-based research, such as our work, which represents the first clinical investigation on this topic within the existing body of literature, hold significant academic value.

CONCLUSION

In peripheral nerve injury, there are some cellular and molecular changes in damaged axon, neuron and also in end-organ. For this reason, factors that will accelerate regeneration are needed in addition to surgical treatment. Exosomes may be used to enhance angiogenesis around the damaged axon, to inhibit scar formation, to regulate immune system by immunomodulatory action and to show trophic effects. In this pilot study, we observed that there was a rapid recovery in a short time without any physical therapy after total radial nerve injury. In the next stage, prospective, randomized and controlled clinical studies on this subject will be needed. Potential future investigations might include the integration of exosomes sourced from stem cells, MSCs, or macrophages with nerve conduit technology or their direct injection into nerve stumps.

ARTICLE HIGHLIGHTS

Research background

Poor functional outcome after surgery is a great challenge in peripheral nerve injury (PNI). Surgical treatments fail to address the complexity of the events that occur following a PNI. There is a clear clinical need to find new approaches. Exosomas from Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs). have the ability to accelerate the improvement of nerve regeneration.

Research motivation

The therapeutic results of exosome application in PNI require investigation.

Research objectives
This study aimed to determine the effects of treatment of peripheral nerve damage with sural autograft and perioperative exosome application.

Research methods
The patient, with total radial nerve injury, underwent a sural autograft repair followed by the per-operative subepineural application of MSC-derived exosome (WJ-MSCs). The patient was monitored for 180 d with neurological examination and electrodiagnostic testing.

Research results
Although physical therapy was not applied after the procedure, improvement in motor and sensory functions occurred in an unusually short time. The nerve exhibited re-innervation signs as evidenced by control electromyography (EMG) at the 10th wk post-injury, and a significant electrophysiological enhancement was observed in the EMG conducted at the 6th-mo follow-up, indicating ongoing regeneration.

Research conclusions
Exosomes (WJ-MSCs) can accelerate the recovery in the treatment of peripheral nerve damage and lead to the improvement in functional outcome.

Research perspectives
New strategies to improve the functional outcome after PNI treatment should be the focus of future studies which can help in increasing the chances of using exosomes clinically for the treatment of PNI.

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FOOTNOTES

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Institutional review board statement: The present study was approved by the medical ethics committee of the authors' institution (protocol number: 56733164-203-E.5863).

Clinical trial registration statement: Our study is a pilot study, not a randomized controlled study. Therefore, the study was not registered. We aim for this study to be a pioneer for the randomized studies we will conduct in the future. We will register the randomized controlled studies we will conduct through the system prior the enrollment.

Informed consent statement: There is human subject in this article and written informed consents were obtained from the patient for their anonymized information to be published in this article and before the stem cell therapies.

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SCIENTOMETRICS

Research progress and challenges in stem cell therapy for diabetic foot: Bibliometric analysis and perspectives

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Abstract

BACKGROUND

Stem cell therapy has shown great potential for treating diabetic foot (DF).

AIM

To conduct a bibliometric analysis of studies on the use of stem cell therapy for DF over the past two decades, with the aim of depicting the current global research landscape, identifying the most influential research hotspots, and providing insights for future research directions.

METHODS

We searched the Web of Science Core Collection database for all relevant studies on the use of stem cell therapy in DF. Bibliometric analysis was carried out using CiteSpace, VOSviewer, and R (4.3.1) to identify the most notable studies.

RESULTS

A search was conducted to identify publications related to the use of stem cells for DF treatment. A total of 542 articles published from 2000 to 2023 were identified. The United States had published the most papers on this subject. In this field, Iran's Shahid Beheshti University Medical Sciences demonstrated the highest productivity. Furthermore, Dr. Bayat from the same university has been an outstanding researcher in this field. *Stem Cell Research & Therapy* is the journal with the highest number of publications in this field. The main keywords were "diabetic foot ulcers," "wound healing," and "angiogenesis."

CONCLUSION

This study systematically illustrated the advances in the use of stem cell therapy to treat DF over the past 23 years. Current research findings suggested that the hotspots in this field include stem cell dressings, exosomes, wound healing, and adipose-derived stem cells. Future research should also focus on the clinical translation of stem cell therapies for DF.

Key Words: Stem cells; Diabetic foot; Bibliometric; CiteSpace; VOSviewer; R-bibliometrix

Core Tip: Through the utilization of bibliometric analysis, this study systematically presented the body of research concerning stem cell therapy in diabetic foot cases, while also identifying focal points and burgeoning trends within this domain.

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INTRODUCTION

Diabetic foot (DF) is one of the most common complications of diabetes. Once infections and recurrences occur in DF patients, wounds are prone to deteriorate, leading to sepsis and an increased risk of amputation[1]. DF constitutes a significant contributor to disability and mortality among individuals with diabetes[2], with as many as 20% of diabetic patients requiring hospitalization due to this condition[3]. Evidence indicates that approximately 4%-10% of individuals with type 2 diabetes may experience DF ulcers (DFUs). Furthermore, the risk of mortality within a 5-year period for those affected by DF is notably elevated, as it is 2.5 times higher than the risk among diabetes patients without DF[4]. It is estimated that a diabetes-related amputation is performed every 20 s globally. The annual mortality rate for DFU patients is as high as 11%, and for amputees, it is as high as 22%[5]. Unfortunately, over the past few decades, there has been no improvement in the incidence and disability rates of DF. Traditional DF therapies have also been unsatisfactory. Moreover, standalone interventions or vascular bypass surgeries are inadequate to address the fundamental pathological mechanisms of widespread blood vessel constriction and blockage in DF as well as nerve and tissue impairments[6].

Currently, most treatment methods for DF mainly target a single factor related to wound healing. Due to advances in regenerative medicine in the clinical setting, there has been widespread interest in the potential impact of stem cells on DF[7]. As a highly promising approach for treating DF[8], the main advantage of stem cell therapy lies in its ability to comprehensively regulate tissue regeneration by improving the microenvironment[9-11]. The mechanisms that contribute to the effectiveness of stem cell therapy involve promoting the deposition of collagen, instigating the formation of new blood vessels, enhancing lower limb blood flow, and mitigating inflammation[12].

Bibliometric analysis, which focuses on the systematic and characteristic features of literature, has been widely used to qualitatively and quantitatively analyze scientific literature[13,14]. It has been extensively applied in gynecology[15], orthopedics[16], complementary and alternative medicine[17], and other medical fields. Bibliometrics serves not only as a tool for comprehensively understanding research trends and hotspots within a particular domain but also as a means to assess the distribution of authors, countries/regions, and journals associated with a specific research field. Therefore, bibliometric analysis lays the foundation for future research directions and development[18]. In this study, a scientific knowledge map of stem cell research within the realm of DF was constructed using CiteSpace, VOSviewer, and R (4.3.1). This approach was employed to examine the hotspots and development trends within this field.

MATERIALS AND METHODS

Publication sources and search methods

For this study, the Web of Science Core Collection (WoSCC) database was selected as the publication source. This database is widely used within the academic community and encompasses a range of internationally renowned scientific journals of significant impact and exceptional quality. It provides a comprehensive and standardized dataset for bibliometric analysis[19]. Due to the dynamic nature of the database, the literature search was performed on 1 d (August 1, 2023) to mitigate potential biases arising from rapid updates. The search strategy for this study is presented in Table 1. The publication types included articles and reviews, and the language limitation was set to English.

Publication screening and access

Two researchers (SHS and YX) independently screened the publications for inclusion in this study. The collected literature was exported in two formats: Complete records and references. They were saved as plain text files under the label "download_txt." The file content included the title, abstract, author information, affiliations, keywords, publication date, and cited references.

Table 1 Literature screening

Set	Publications	Screen
1	597	Topic: (TS=("Stem Cells" OR "Cell, Stem" OR "Cells, Stem" OR "Stem Cell" OR "Progenitor Cells" OR "Cell, Progenitor" OR "Cells, Progenitor" OR "Progenitor Cell" OR "Mother Cells" OR "Cell, Mother" OR "Cells, Mother" OR "Mother Cell" OR "Colony-Forming Unit" OR "Colony Forming Unit" OR "Colony-Forming Units" OR "Colony Forming Units")) AND TS=("Foot, Diabetic" OR "Diabetic Feet" OR "Feet, Diabetic" OR "Foot Ulcer, Diabetic" OR "Diabetic Foot")
2	547	Types of publications: (ARTICLES OR REVIEWS)
3	543	Languages of publications: (ENGLISH)

Data analysis

All pertinent documents retrieved from the WoSCC database were imported into bibliometrix (based on R 4.3.1.), VOSviewer, and CiteSpace for visualization analysis. Two essential metrics commonly employed to evaluate research performance are the number of publications (Np), which serves as a gauge of productivity, and the number of citations (Nc), which serves as an indicator of impact.

R-bibliometrix is used to conduct bibliometric analysis on leading research nations, institutions, and journals[20]. The primary application of the h-index is to assess researchers' academic contributions and anticipate their forthcoming scientific accomplishments. The g-index, which is derived from the h-index, can further measure the impact and scholarly achievements of researchers[21]. Moreover, these indices can be obtained to characterize the publication output of a country or region as well as the output of an institution or journal[19,22].

VOSviewer is a network analysis software for scientometric research that was developed by the Centre for Science and Technology Studies at Leiden University in the Netherlands. It provides visual analysis and allows the creation of maps based on network data. The software enables connections between items through cocitation links, co-occurrence, citations, and bibliographic coupling. VOSviewer offers three types of visual maps: Network; overlay; and density visualization[23,24].

The CiteSpace software is a citation visualization and analysis tool rooted in scientometrics and data visualization. It was created by Professor Chaomei Chen at Drexel University using the Java programming language[25]. By leveraging data mining, information analysis, and map visualization techniques, it effectively illustrates the architecture, patterns, and dissemination of scientific knowledge[26].

RESULTS

An overview of stem cells in DF publications

After retrieving and filtering publications from the WoSCC database, a total of 543 publications were included. Among them, 350 articles and 193 reviews were ultimately included in this analysis. The studies were published between 2000 and 2023, and the h-index was 71.

Figure 1 illustrates the geographic distribution of the overall research paper count across all countries and regions. Among the 543 articles, the top two countries accounted for more than half of the total. The United States had the highest number of published papers, followed by China, Iran, England, and Italy.

Between 2000 and 2022, the annual Np increased rapidly and exhibited a polynomial fit, $y = 0.1484x^2 - 593.9x + 594231$, $R^2 = 0.9714$. The annual cumulative publication volume followed an exponential curve, $y = 2E\text{-}247e^{0.2843x}$, $R^2 = 0.9601$ (Figure 2).

Country/region contributions to global publications

The top 10 countries/regions in terms of output are shown in Table 2. The country with the highest number of published papers was the United States (183/543, 33.70%), followed by China (152/543, 27.99%), Iran (35/543, 6.45%), England (33/543, 6.08%), and Italy (28/543, 5.16%). Papers from the United States were cited 9663 times, accounting for 44.84% of the total citations, followed by China (3828; 17.76%) and England (2449; 11.36%). In addition, the United States (46) had the highest h-index, followed by China (32) and England (20). The network (Figure 3A) and density (Figure 3B) maps constructed using VOSviewer also indicated the research influence of the United States and China. As shown in Figure 3C, the largest connected component in the co-occurrence network of countries/regions consisted of 60 nodes and 185 connections (density = 0.01). The purple hue corresponds to the betweenness centrality coefficients of countries/regions, encompassing the United States (0.83), England (0.23), Italy (0.17), and China (0.15). This indicates that these countries/regions assume a "bridging" role within this domain, and this finding was also confirmed by the multiple-country publication value in Figure 3D.

Analysis of author institution publications

We compiled a list of the 10 institutions with the highest Np (Table 3). These institutions were located in the United States (5/10), China (4/10), and Iran (1/10). The top six institutions in terms of publication ranking included Shahid Beheshti University Medical Sciences (Iran, 13/2.39%), University of Louisville (the United States, 12/2.21%), Army Medical University (China, 12/2.21%), Tongji University (China, 11/2.03%), Chinese Academy of Medical Sciences-Peking Union

Table 2 Top 10 countries/regions with the highest research productivity

No.	Country	Np	Np, %	h-index	Nc	Nc, %	Centrality
1	United States	183	33.70	46	9663	44.84	0.83
2	China	152	27.99	32	3828	17.76	0.15
3	Iran	35	6.45	16	901	4.18	0.03
4	England	33	6.08	20	2449	11.36	0.23
5	Italy	28	5.16	14	721	3.35	0.17
6	India	25	4.60	11	1249	5.80	0.02
7	Germany	24	4.42	13	766	3.55	0.05
8	South Korea	16	2.95	11	953	4.42	0.06
9	Spain	16	2.95	8	362	1.68	0.02
10	Ireland	13	2.39	8	981	4.55	0.09

Np: Number of publications; Nc: Number of citations.

Table 3 Top 10 most productive affiliations

No.	Institutions	Country	Np	Np/N1, %	h-index	Nc	Nc, %	Centrality
1	Shahid Beheshti University Medical Sciences	Iran	13	2.39	8	241	0.01	0
2	University of Louisville	United States	12	2.21	7	160	0.01	0
3	Army Medical University	China	12	2.21	11	331	0.02	0
4	Tongji University	China	11	2.03	10	499	0.02	0.02
5	Chinese Academy of Medical Sciences-Peking Union Medical College	China	11	2.03	6	272	0.01	0.04
6	Boston University	United States	11	2.03	9	813	0.04	0.13
7	University of California System	United States	10	1.84	10	386	0.02	0.13
8	Harvard University	United States	10	1.84	12	909	0.04	0.06
9	University of Miami	United States	9	1.66	8	859	0.04	0
10	Shanghai Jiao Tong University	China	7	1.29	4	191	0.01	0.06

Np: Number of publications; Nc: Number of citations.

Table 4 Top 5 authors and cocited authors with the most publications

No.	Ref.	Np	h-index	Institution	Country	Cocited author	Centrality	Cocitation
1	Bayat *et al*[28]	12	7	Shahid Beheshti University of Medical Sciences	Iran	FALANGA V	0.36	151
2	Amini *et al*[29]	12	7	Shahid Beheshti University of Medical Sciences	Iran	BREM H	0.12	87
3	Tomic-Canic *et al*[30]	12	3	University of Miami	United States	ARMSTRONG DG	0.21	86
4	Veves *et al*[31]	6	7	Harvard Medical School	United States	BOULTON AJM	0.06	70
5	Pastar *et al*[32]	4	6	University of Miami	United States	MARSTON WA	0.10	59

Np: Number of publications.

Medical College (China, 11/2.03%), and Boston University (density = 0.01). Boston University (0.13) and the University of California System (0.13) had the highest centrality, and their nodes were identified by purple circles (Figure 4).

Author analysis and cocitation author analysis.

In 1997, economists Katz and Martin provided a definition for "scientific collaboration," wherein scholars unite to collab-

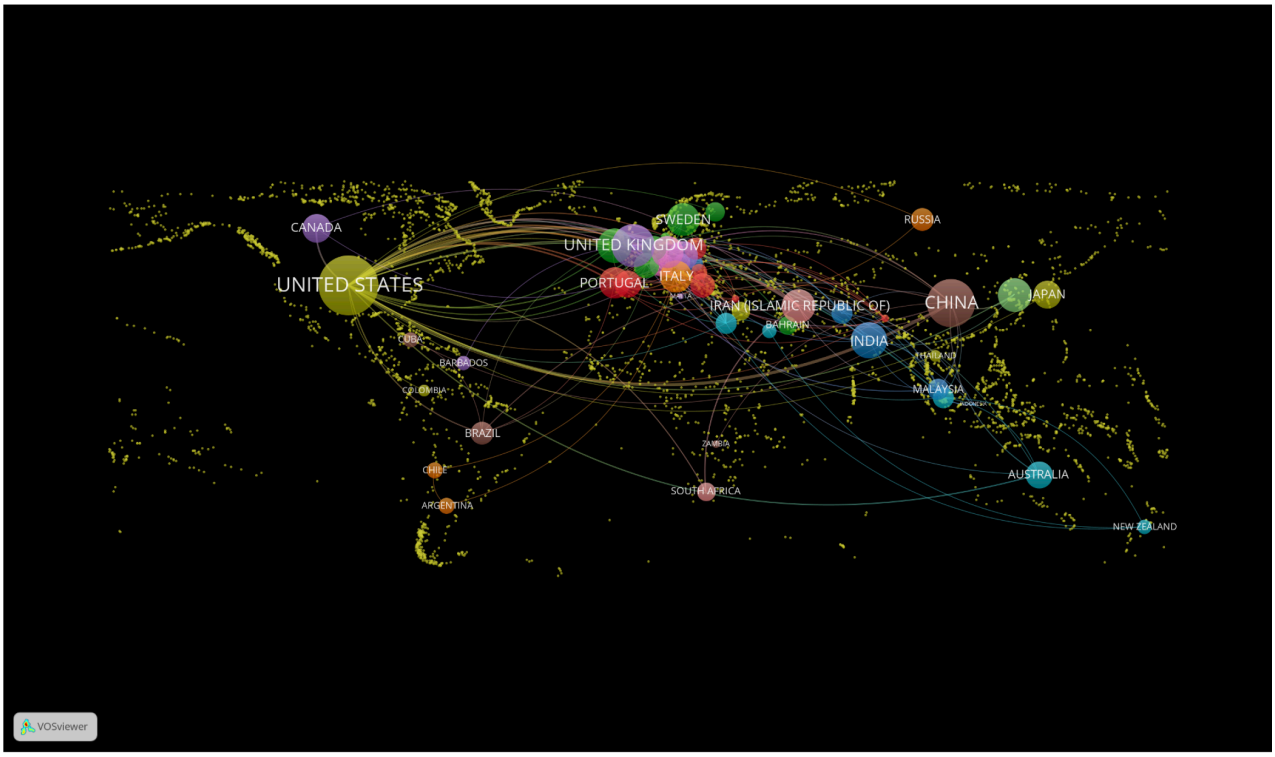

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Figure 1 Geographical distribution of publications on stem cells in diabetic foot research.

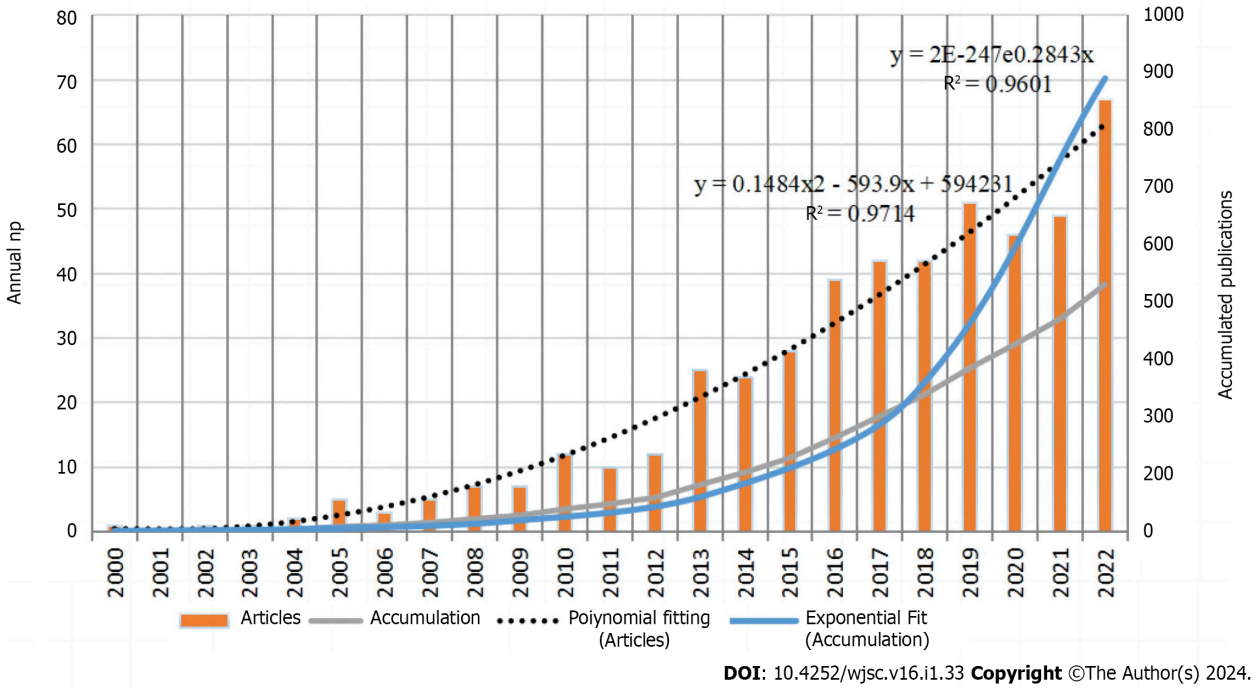

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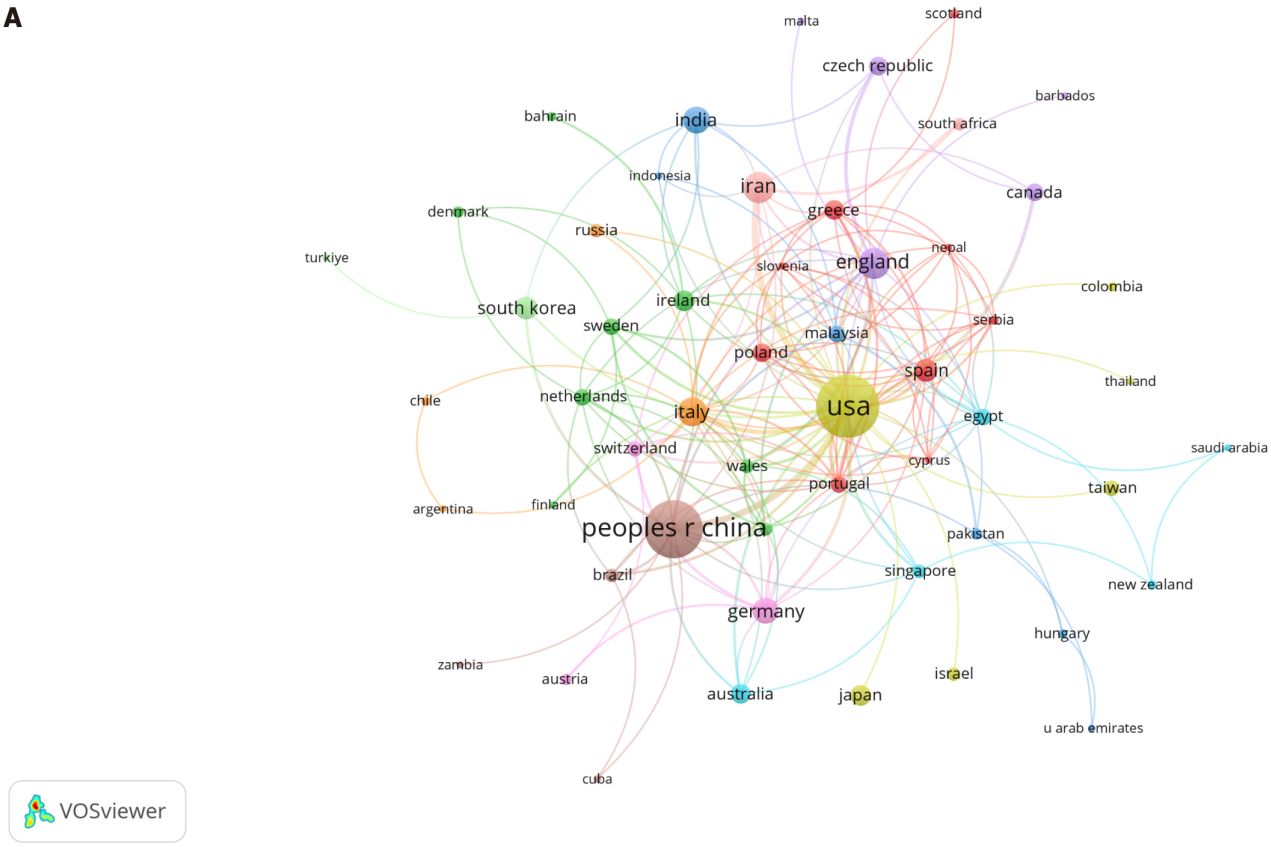
Figure 2 Publication count divided by year over the past 23 years. Np: Number of publications.

oratively pursue shared scientific objectives[27]. We identified 2798 authors who have published articles on the topic of stem cells in DF research. Figure 5A displays the 10 authors with the highest average annual publication count. Among them, Bayat[28], Amini[29], and Tomic-Canic[30] published the highest number of studies (n = 12), followed by Veves (n = 6)[31] and Pastar C (n = 4)[32] (Table 4). Cocitation authors refer to the authors who are cited together in the same article. Figure 5B and C show the network and density maps of cocited authors, respectively. Among the 28753 cocited authors, 173 have been cited together more than 20 times. The top five cocited authors were FALANGA V (n = 151), BREM H (n = 87), ARMSTRONG DG (n = 86), BOULTON AJM (n = 70), and MARSTON WA (n = 59) (Table 4). As shown

A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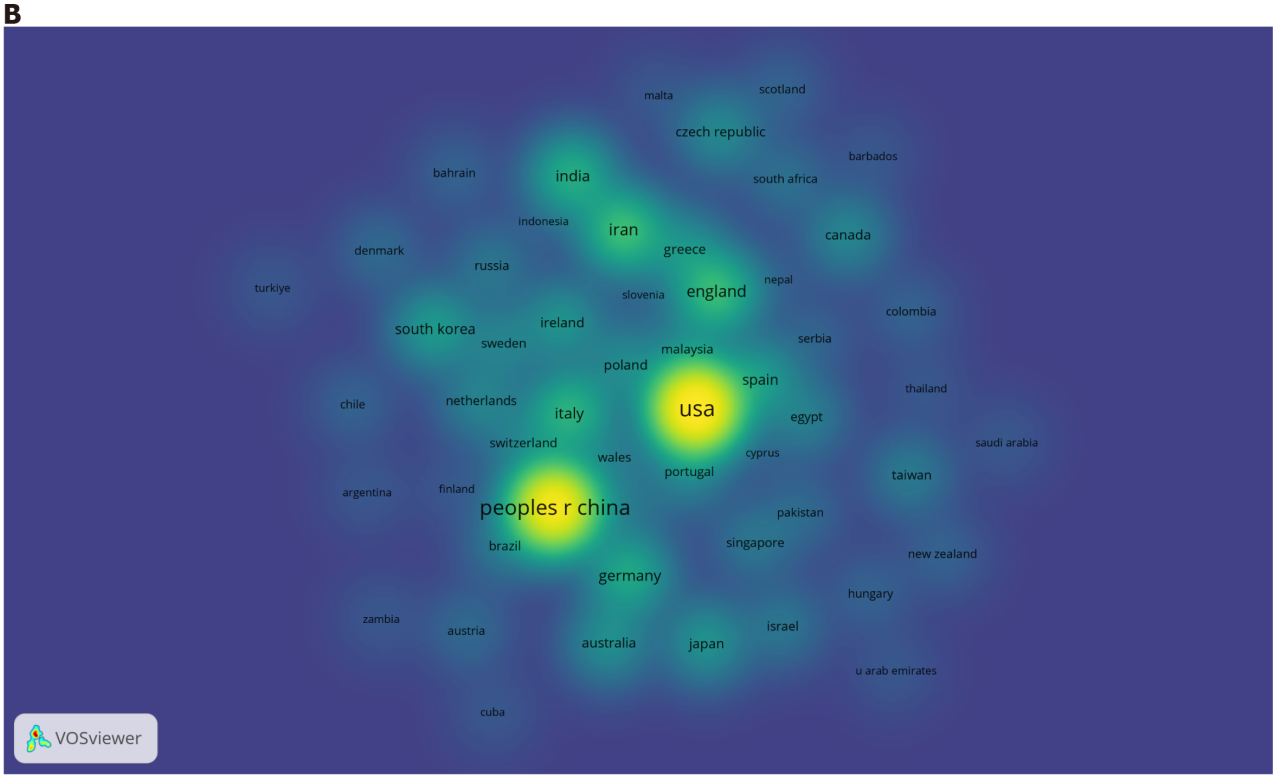
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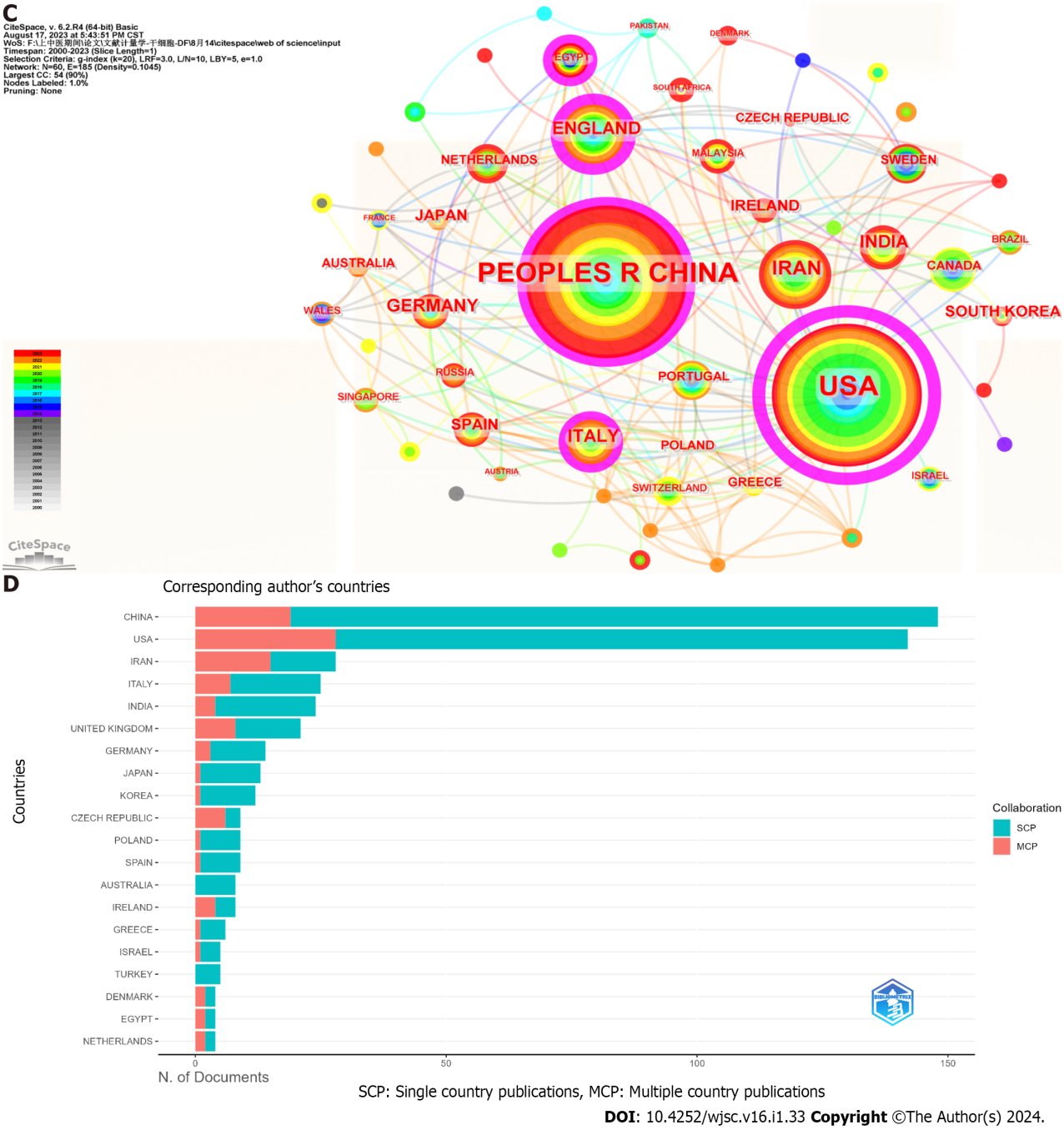

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Figure 3 Contributions of different countries to research on stem cells in the diabetic foot field. A: Network visualization of country collaboration; B: Density map of cooperation between countries; C: A network diagram showing international collaborations, with purple circles representing intermediation centrality; D: Top 20 countries for corresponding authors. MCP: Multiple-country publications; SCP: Single-country publications.

in Figure 5D, the author with the highest betweenness centrality in terms of citations was FALANGA V (0.36), followed by ARMSTRONG DG (0.21) and BOULTON AJM (0.12).

Journals and cocited journals

Studies related to the use of stem cell therapy in DF patients were published in a total of 280 different journals. The journal *Stem Cell Research & Therapy* [22/7.86%, impact factor (IF): 7.5, Journal Citation Reports (JCR): Q1] had the highest output quantity; this journal emphasizes basic, clinical, and translational research on stem cell therapy and regenerative medicine. The next most productive journal was *International Wound Journal* (17/6.07%, IF: 3.1, JCR: Q2). Among the top 10 journals, 8 belonged to the JCR Q1 category, with 9 having an IF exceeding 3 (Table 5). Additionally, *Stem Cell Research & Therapy* had the highest h-index (Figure 6A) and g-index (Figure 6B).

Among the 4667 cocited journals, 82 journals have been cited more than 100 times. Table 2 shows that *Wound Repair and Regeneration* (1297/27.79%, IF: 2.9, JCR: Q1) had the highest number of citations, followed by *Diabetes Care* (951/20.38%, IF: 16.2, JCR: Q1) and *Biomaterials* (681/14.59%, IF: 14, JCR: Q1). Among the top 10 cocited journals, 8 were in JCR Q1, and they all had an IF exceeding 3 (Table 5 and Figure 6C).

Table 5 Top 10 journals and cocited journals with the most publications

No.	Journal	Np	IF in 2020	JCR	Cocited journal	Cocitation, %	IF in 2020	JCR
1	*Stem Cell Research & Therapy*	22	7.5	Q1	*Wound Repair and Regeneration*	1297 (27.79)	2.9	Q1
2	*International Wound Journal*	17	3.1	Q2	*Diabetes Care*	951 (20.38)	16.2	Q1
3	*Wound Repair and Regeneration*	14	2.9	Q1	*Biomaterials*	681 (14.59)	14.0	Q1
4	*Advances in Wound Care*	11	4.9	Q1	*Journal of Investigative Dermatology*	668 (14.31)	6.5	Q1
5	*Diabetes-Metabolism Research and Reviews*	9	8.0	Q2	*PLOS One*	626 (13.41)	3.7	Q2
6	*International Journal of Molecular Sciences*	9	5.6	Q1	*Plastic and Reconstructive Surgery*	576 (12.34)	3.6	Q1
7	*Frontiers in Endocrinology*	8	5.2	Q1	*International Wound Journal*	575 (12.32)	3.1	Q2
8	*Biomedicines*	8	4.7	Q1	*Stem Cell Research & Therapy*	488 (10.46)	7.5	Q1
9	*Plastic and Reconstructive Surgery*	8	3.6	Q1	*Stem Cells*	462 (9.90)	5.2	Q1
10	*Diabetes*	7	7.7	Q1	*Advances in Wound Care*	457 (9.79)	4.9	Q1

IF: Impact factor; JCR: Journal Citation Reports; Np: Number of publications.

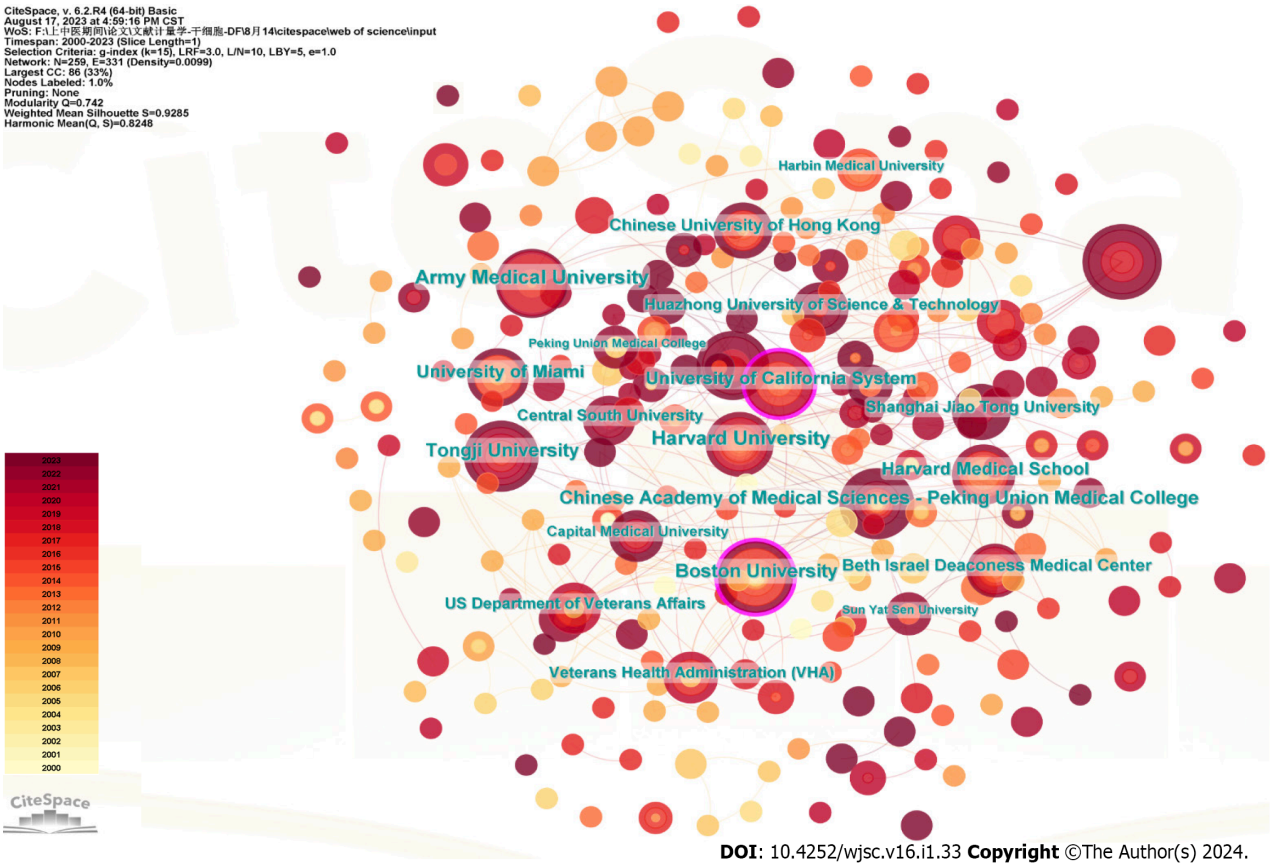

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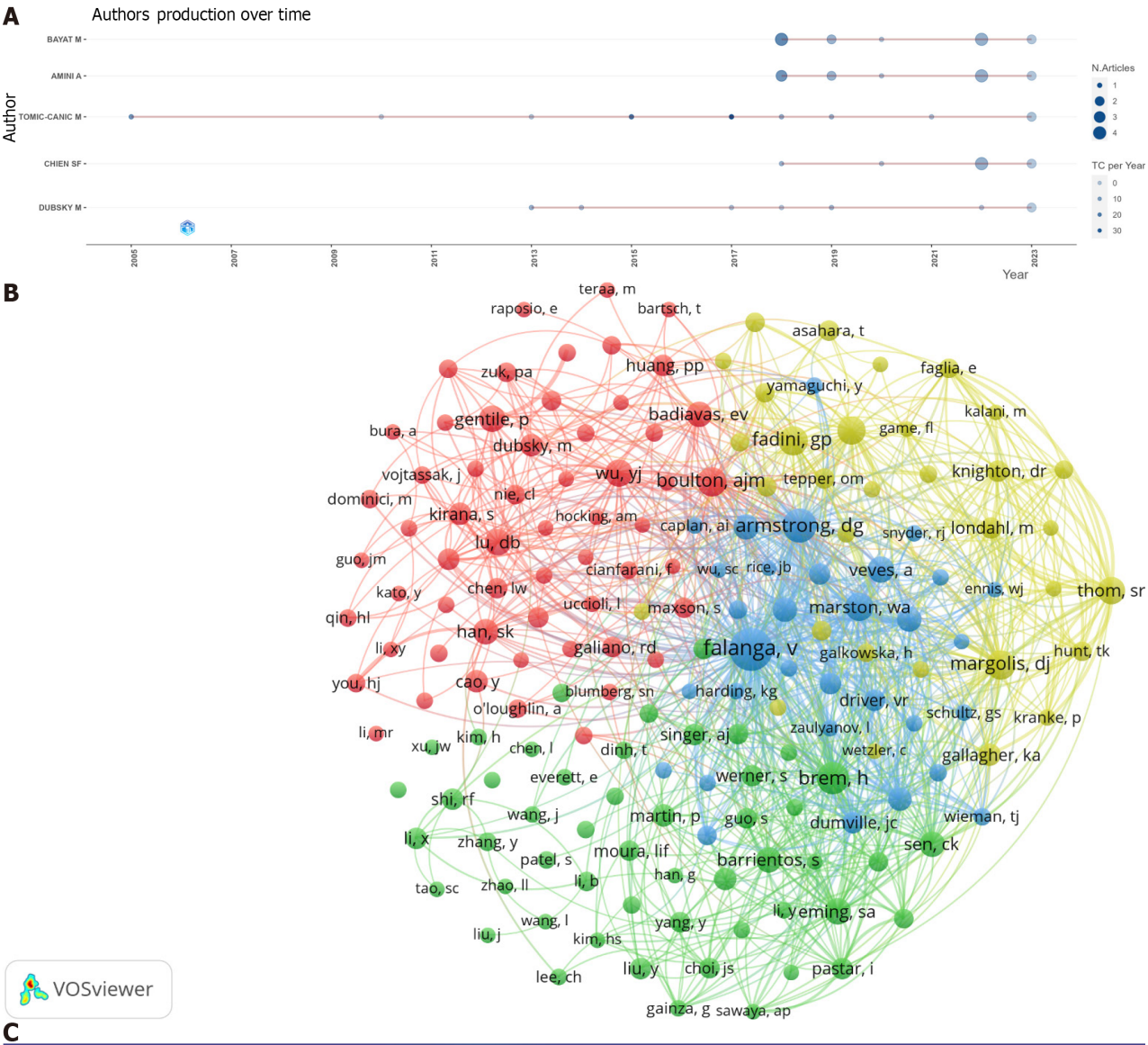
Figure 4 Institutional collaboration network diagram, with purple circles representing intermediation centrality.

The dual plot of the journals displays the distribution of relationships between them. As shown in Figure 6D, there are primarily four citation pathways, consisting of two orange paths and two green paths. The relevant pathways are listed in Table 6.

Cocited reference analysis

Cocitation describes how often two documents are referenced jointly[33]. Table 7 lists the top 10 referenced articles that were most commonly cocited. Among the 28717 cited articles, 27 articles have been cited more than 30 times. The top six referenced articles have all been cited more than 50 times. The article by Armstrong *et al*[34] that was published in the *New England Journal of Medicine* in 2017 had the highest number of cocitations, followed by the article published by Lopes *et al*[35] in 2018 (Figure 7A). Based on cluster analysis, a total of eight clusters were identified (Figure 7B), indicating the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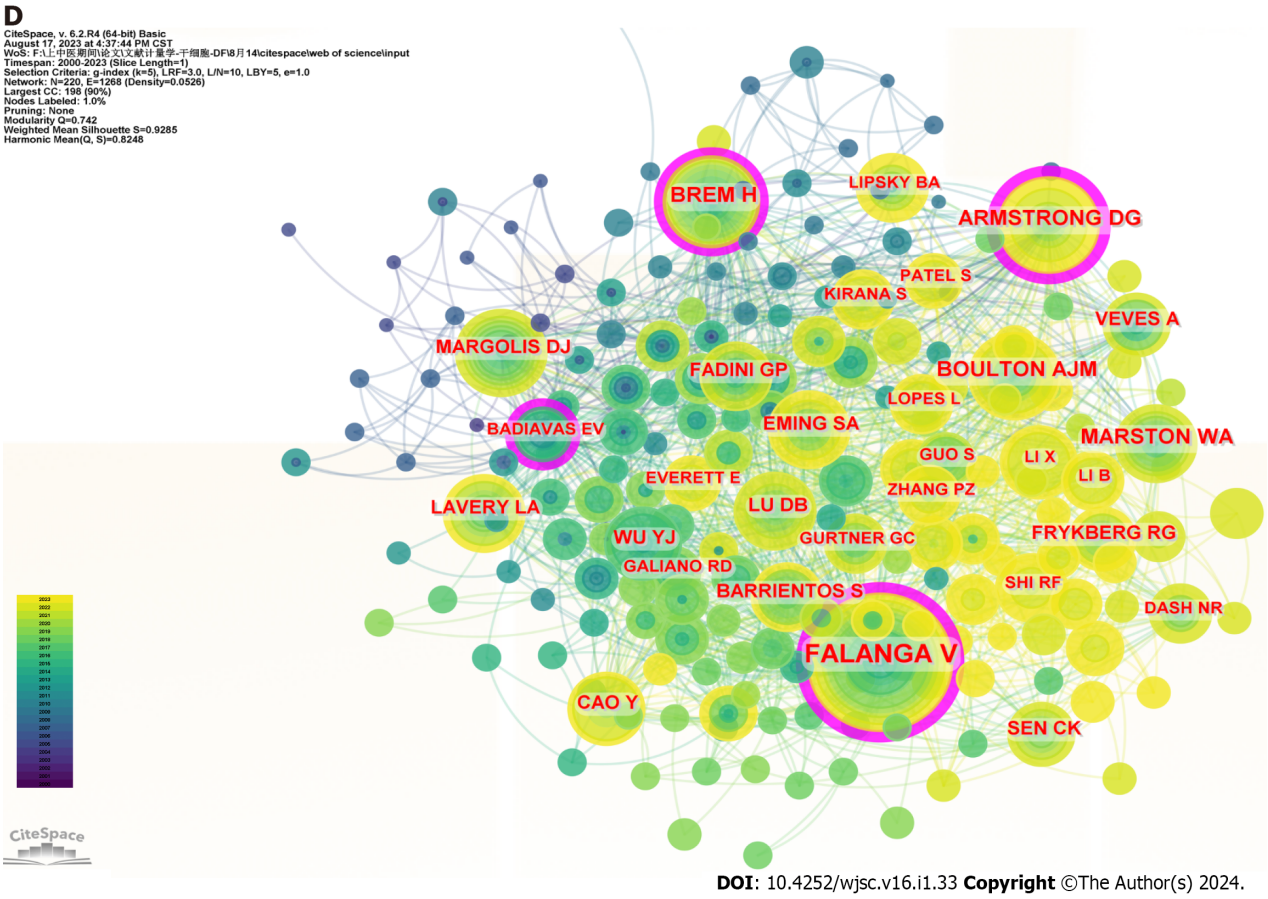

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Figure 5 Contributions of different authors to research on stem cells in the diabetic foot field. A: Top 10 authors in terms of average publication count per year; B: Network visualization of country collaboration; C: Density map of cooperation between countries; D: Network diagram showing international collaborations, with purple circles representing intermediation centrality.

reliability of the clustering results. These clusters primarily included #0 "diabetic foot," #1 "skin defect," #2 "low-level laser therapy," #3 "adipose-derived stem cell therapy for local chronic radiation injury," #4 "occlusive dressing," and #5 "exosomes." In addition, research hotspots can be reflected in the timeline of cocited references. Relatively speaking, cluster #0 "diabetic foot" and cluster #5 "exosomes" were recent hotspots. Furthermore, analysis of citation bursts can accurately identify articles that have garnered significant attention within a particular field. This method can help filter out articles that are likely to have a major impact on future research. The initial instance of a powerful citation burst emerged in 2004[36], whereas the latest occurrence of a significant citation burst appeared in 2020[37]. Additionally, the study published by Maxson *et al*[38] in 2012 exhibited the strongest citation burst intensity (10.19)[39] (Figure 7C).

Analysis of co-occurrence keywords

A total of 2380 keywords were derived from the included studies. After excluding keywords that appeared fewer than five times and merging equivalent keywords, a total of 219 keywords were identified. We have displayed the top 20 keywords (Table 8) and built a related network (Figure 8A) and density graphs (Figure 8B). The top five keywords were "diabetic foot ulcers" (253), "wound healing" (130), "mesenchymal stem cells" (98), "stem cells" (78), and "angiogenesis" (75). Figure 8 displays the keyword timeline, illustrating the timeline of keywords in the clusters based on their appearance dates. The color of the keywords matches the cluster label color (Figure 8C). A total of eight clusters were identified: #0 "diabetes mellitus;" #1 "expression;" #2 "chronic wounds;" #3 "adipose-derived stem cells;" #4 "diabetic foot;" #5 "diabetic wound healing;" #6 "double-blind;" and #7 "biological therapies." Additionally, Figure 8D displays the top 10 keywords with the highest citation bursts. "Repair" (5.15) had the highest burst strength, followed by "skin" (4.15) and "venous leg ulcers" (3.93). "Endothelial progenitor cells" (2006-2014) and "venous leg ulcers" (2015-2019) have shown prolonged citation bursts, indicating that research in these areas has been attracting an increasing amount of attention from researchers.

DISCUSSION

Stem cell therapy has recently emerged as a novel approach for DF management, as it has exhibited safety and efficacy across preclinical and clinical trials[35]. This study is the first bibliometric analysis on global research related to the use of stem cell therapy for DF. These findings can provide researchers with a systematic and intuitive overview of the overall

Table 6 Paths between citing journals and cited journals

No.	Journal	Cited journal	Path color
1	*Molecular/Biology/Immunology*	*Molecular/Biology/Genetics*	Orange
2	*Molecular/Biology/Immunology*	*Health/Nursing/Medicine*	Orange
3	*Medicine/Medical/Clinical*	*Molecular/Biology/Genetics*	Green
4	*Medicine/Medical/Clinical*	*Health/Nursing/Medicine*	Green

Table 7 Top 10 cocited references referring to cocitations

Ref.	Year	Journal	DOI	Cocitations	Centrality
Armstrong *et al*[34]	2017	*New England Journal of Medicine*	10.1056/NEJMra1615439	32	0.42
Lopes *et al*[35]	2018	*Stem Cell Research & Therapy*	10.1186/s13287-018-0938-6	26	0.09
Li *et al*[61]	2018	*Experimental & Molecular Medicine*	10.1038/s12276-018-0058-5	25	0.02
Everett and Mathioudakis[62]	2018	*Annals of the New York Academy of Sciences*	10.1111/nyas.13569	25	0.09
Cao *et al*[63]	2017	*Journal of Diabetes Research*	10.1155/2017/9328347	23	0.10
Patel *et al*[64]	2019	*Biomedicine & Pharmacotherapy*	10.1016/j.biopha.2019.108615	22	0.04
Moon *et al*[65]	2019	*Diabetes*	10.2337/db18-0699	20	0.06
Maxson *et al*[38]	2012	*Stem Cells Translational Medicine*	10.5966/sctm.2011-0018	19	0.10
Zhang *et al*[39]	2017	*Annals of Medicine*	10.1080/07853890.2016.1231932	18	0.06
Li *et al*[66]	2020	*About Molecular Therapy – Nucleic Acids*	10.1016/j.omtn.2019.11.034	16	0.02

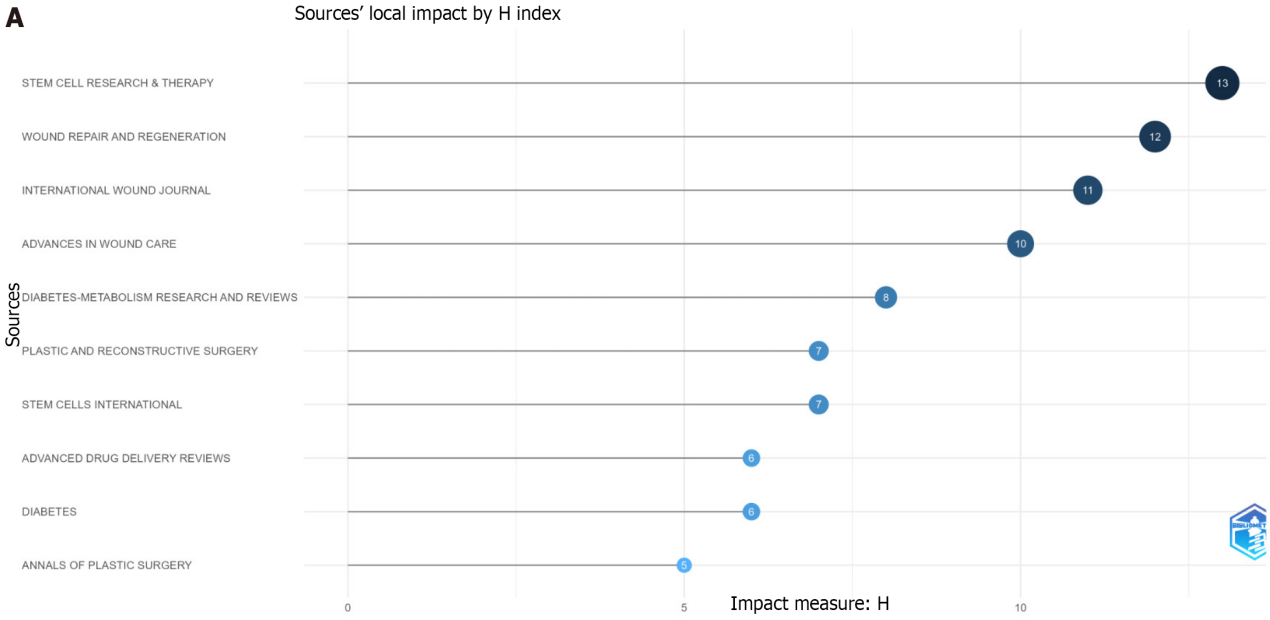
Table 8 Top 20 keywords based on their frequency

No.	Occurrence frequency	Centrality	Year	Keywords	No.	Occurrence frequency	Centrality	Year	Keywords
1	253	0.51	2004	Diabetic foot ulcers	11	47	0.04	2007	Expression
2	119	0.04	2011	Wound healing	12	44	0.12	2005	Chronic wounds
3	98	0.05	2013	Mesenchymal stem cells	13	40	0.02	2013	Stem cells
4	78	0.04	2011	Stem cells	14	38	0.08	2004	Diabetes mellitus
5	75	0.13	2006	Angiogenesis	15	37	0.03	2005	Skin
6	71	0.06	2005	Diabetic foot	16	34	0.04	2012	Critical limb ischemia
7	57	0.06	2011	Therapy	17	33	0.03	2011	*In vitro*
8	55	0.09	2004	Management	18	32	0.02	2011	Stromal cells
9	51	0.12	2006	Endothelial progenitor cells	19	31	0.03	2014	Proliferation
10	48	0.05	2004	Differentiation	20	30	0.02	2011	Foot ulcers

trends in this field[40,41].

General information

Based on the information retrieved from the WoSCC database as of August 1, 2023, there have been a total of 982 studies related to stem cell therapy and DF published across 280 academic journals. These studies involve a total of 2798 authors affiliated with 543 institutions across 60 countries/regions. The yearly fluctuations in Np serve as a significant gauge for discerning development trends within this domain[42,43]. The publication trends from 2000 to 2003 indicate a lack of research during this period, suggesting a limited depth of study on stem cell therapy in DF. The year 2004 was a turning point for this topic[44], as an increasing number of researchers started focusing on the role of stem cell therapy in the treatment of DF. At that time, the number of relevant publications began to show a rapid upward trend.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ADVANCES IN WOUND CARE
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PLASTIC AND RECONSTRUCTIVE SURGERY
STEM CELLS INTERNATIONAL
ADVANCED DRUG DELIVERY REVIEWS
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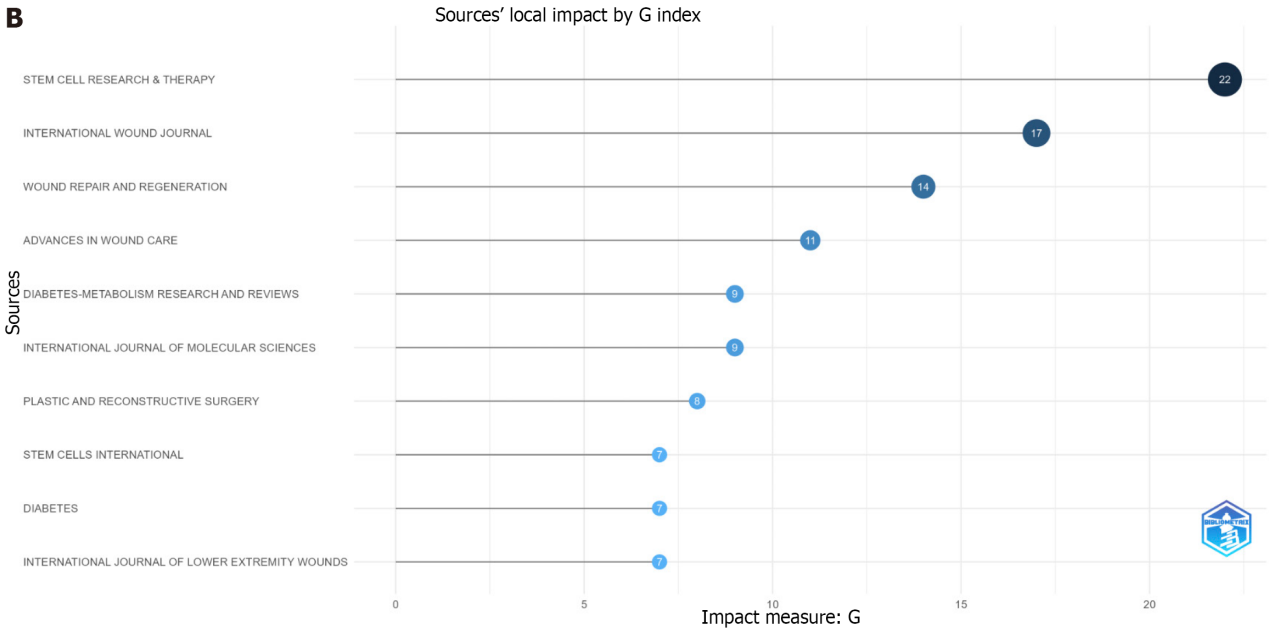
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INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES
PLASTIC AND RECONSTRUCTIVE SURGERY
STEM CELLS INTERNATIONAL
DIABETES
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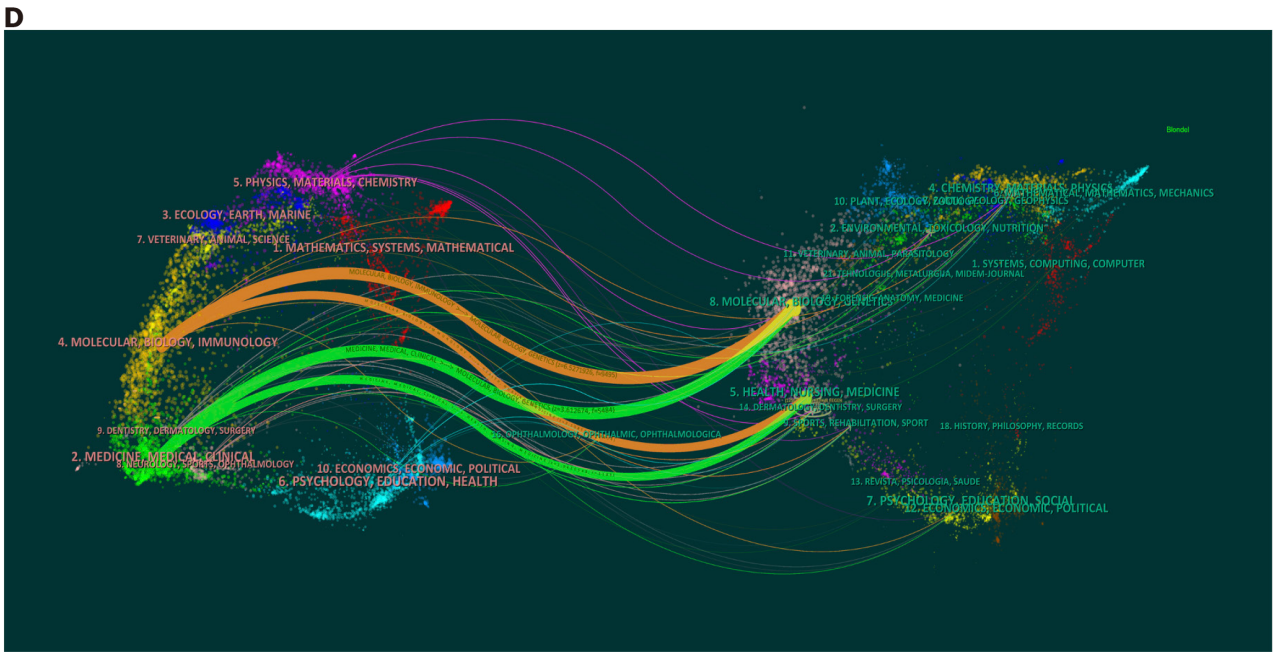
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3. ECOLOGY, EARTH, MARINE
7. VETERINARY, ANIMAL, SCIENCE
1. MATHEMATICS, SYSTEMS, MATHEMATICAL
4. MOLECULAR BIOLOGY, IMMUNOLOGY
9. DENTISTRY, DERMATOLOGY, SURGERY
2. MEDICINE, MEDICAL, CLINICAL
10. ECONOMICS, ECONOMIC, POLITICAL
6. PSYCHOLOGY, EDUCATION, HEALTH
4. CHEMISTRY, MATERIALS, PHYSICS
2. ENVIRONMENTAL, TOXICOLOGY, NUTRITION
1. SYSTEMS, COMPUTING, COMPUTER
8. MOLECULAR BIOLOGY, GENETICS
5. HEALTH, NURSING, MEDICINE
18. HISTORY, PHILOSOPHY, RECORDS
7. PSYCHOLOGY, EDUCATION, SOCIAL

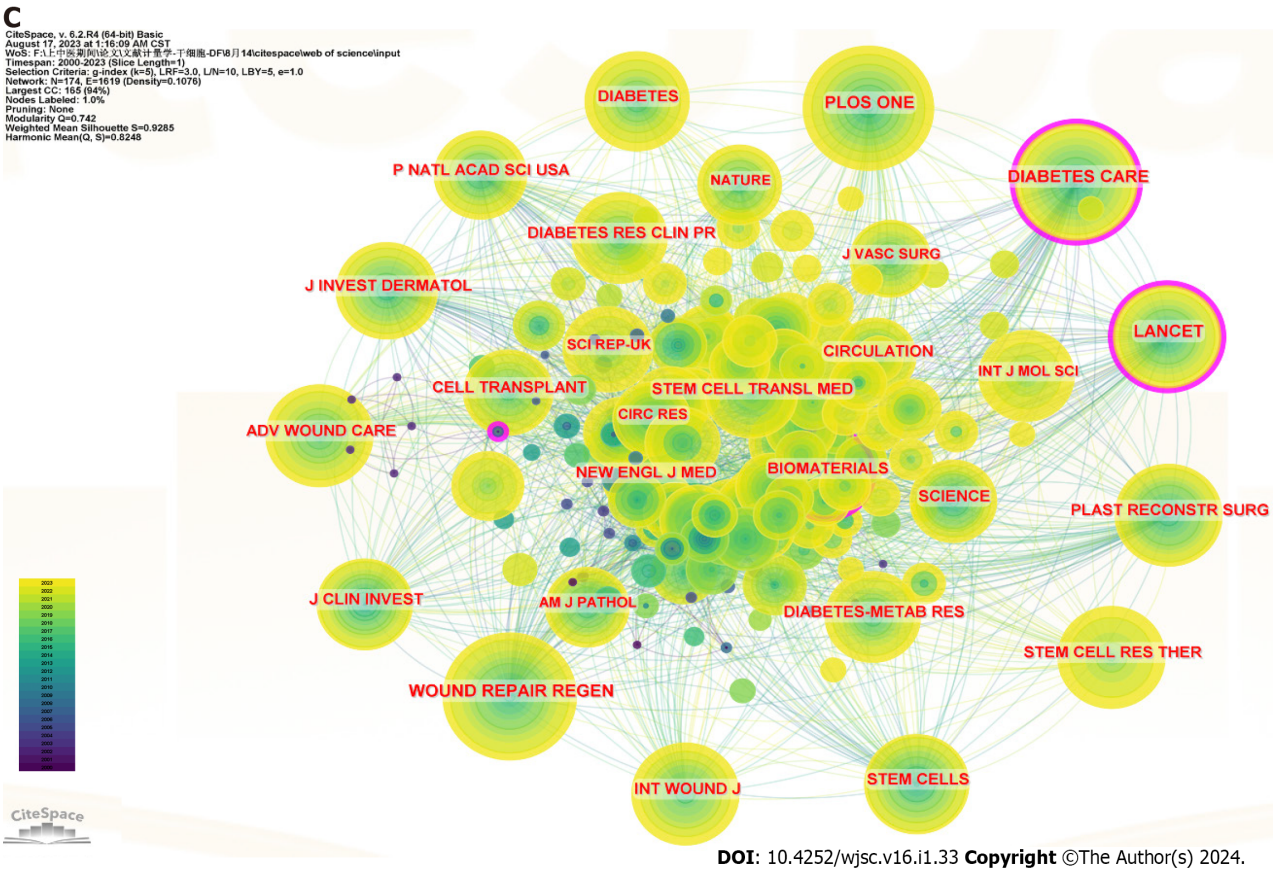

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Figure 6 Contributions of different authors to research on stem cells in the diabetic foot field. A: Top 10 journals in terms of h-index; B: Top 10 journals in terms of g-index; C: CiteSpace visualization of cocited journals; D: Biplot overlay of journals on stem cells in the diabetic foot field (left side represents areas covered by citing journals, and the right side represents areas covered by cited journals).

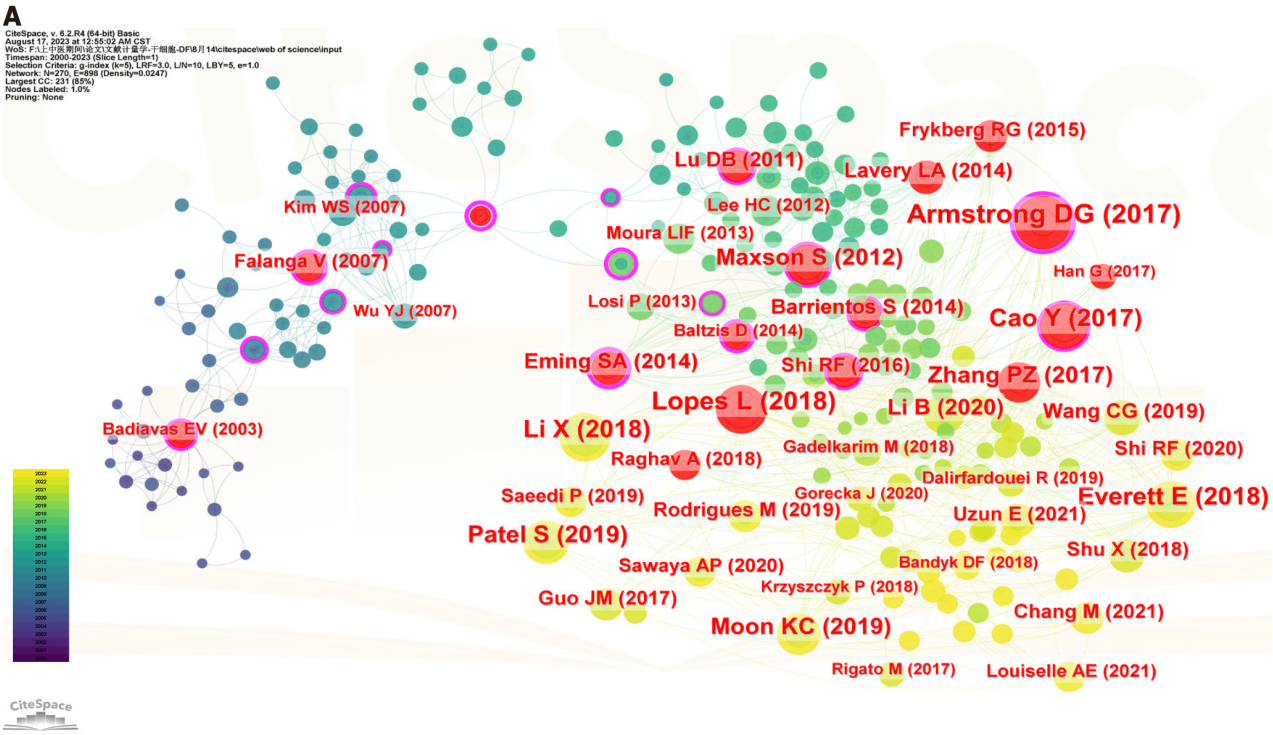
Through visual analysis of national and institutional distribution, we can see that the United States and China are the leading countries in the research and development of stem cell therapy for DF. Among the top 10 institutions researching this topic, the majority (90%) are located in the United States and China, including the University of Louisville, Army Medical University, and Tongji University. Within the 10 highest-ranked nations, the United States (0.83) demonstrated the strongest centrality and maintained the highest multiple-country publication value, signifying its substantial impact within this area. Other countries, such as China (0.15), England (0.23), and Italy (0.17), also had centrality values exceeding 0.1, suggesting their involvement in international exchanges and collaborations to a certain extent.

Identifying the core authors in this field can help researchers find potential collaborators[45]. Professor Bayat, from Shahid Beheshti University of Medical Sciences in Iran, was the author with the highest number of published papers. He has conducted a series of studies on the mechanisms and therapeutic effects of photobiomodulation in stem cell therapy for DF[46,47]. His research has shown that subjecting diabetic adipose-derived stem cells to photobiomodulation prior to treatment markedly expedites the process of wound healing[48]. Among the cited authors, Vincent Falanga from Boston University School of Medicine has been referenced 151 times and had the largest node in this field. He has conducted several high-quality reviews on the use of stem cells for the treatment of chronic wounds[49,50].

The analysis of the distribution of academic journals helps to identify the core journals in specific research fields[51]. Multiple studies on the use of stem cells in DF have been published in influential journals such as *Stem Cell Research & Therapy* and the *International Wound Journal*. Among the top 10 journals, 8 are classified as JCR Q1, which indicates that the research quality of articles in the field is high. From the perspective of commonly cited academic journals, we can see that most of the research comes from highly influential journals in the field of stem cells or wound repair. The journal dual overlay represents the thematic distribution of academic journals, with four citation paths. This implies that current research related to stem cells and DF is focused not only on basic research but also on translational medical research.

Intellectual base

Analysis of cocited references can provide in-depth insights into the core themes and major discoveries of current research[52]. The primary emphasis of the top 10 most cocited references revolves around the pathological and physiological mechanisms, treatment approaches, and everyday handling of stem cells in the context of DF. The results of citation analysis revealed that the article written by Armstrong *et al*[34] published in the *New England Journal of Medicine* in 2017 had the highest frequency of cocitations. This article described the epidemiology and health management of recurrent DFUs and emphasized that the focus of work should be on prevention. Another study published by Lopes *et al* [35] in 2018, which has a citation count of 32, proposed that stem cell therapy was an effective method for treating DFUs.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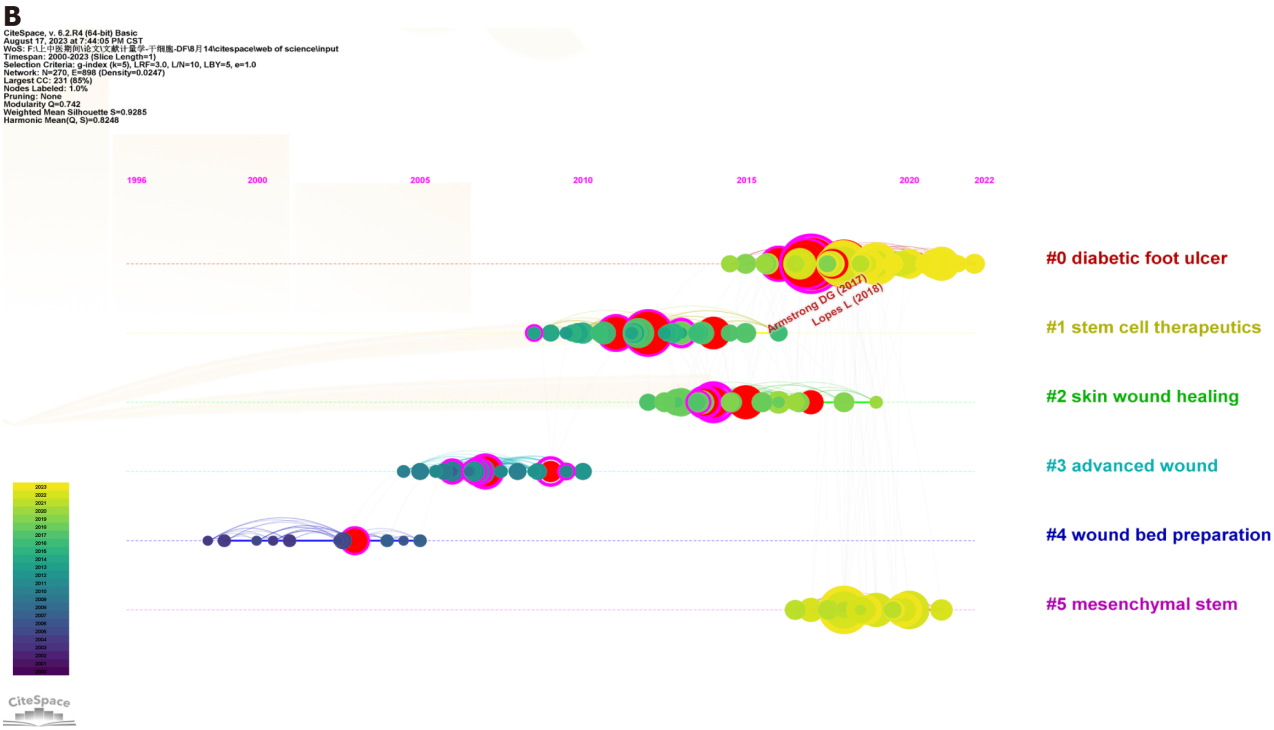
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#2 skin wound healing
#3 advanced wound
#4 wound bed preparation
#5 mesenchymal stem
Armstrong DG (2017)
Lopes L (2018)
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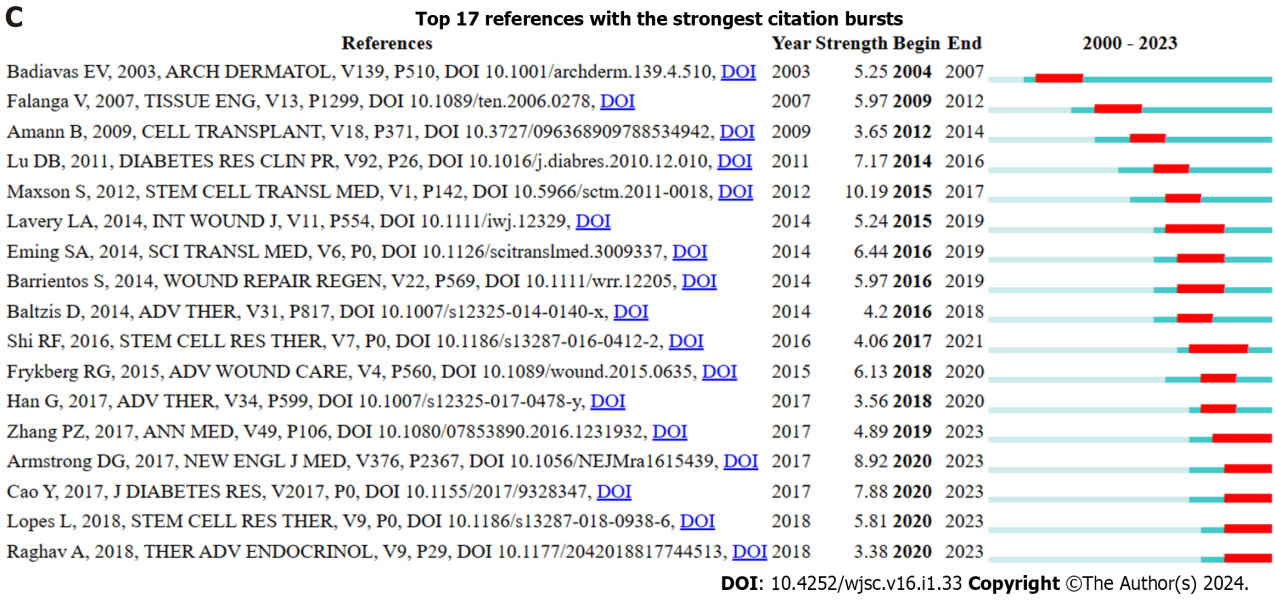


Figure 7 Analysis of cocited references. A: Visualization of cocited references. Nodes represent cocited references, with red circles representing citation bursts references; B: Timeline graph of cluster analysis; C: Top 17 references with the strongest citation bursts.

This suggested that for some patients who do not have other options for vascular reconstruction, stem cell therapy can be considered an alternative to amputation. The most explosive reference was the paper published by Maxson *et al*[38] in *Stem Cells Translational Medicine*. This article delineated the function of mesenchymal stem cells (MSCs) in the process of wound healing and elucidated their ability to attract additional host cells and release growth factors and matrix proteins to orchestrate the mending mechanism.

In the clustering analysis of cocited references, there were modules related to occlusive dressing, low-level laser, and exosomes, among others. With the use of advanced techniques, there have been some breakthroughs in the research on stem cells in the field of DF science. Exosomes derived from MSCs carry forward the robust functions of their originating cells. These functions include tasks such as managing inflammation and immune responses, fostering angiogenesis, facilitating cell proliferation and movement, mitigating oxidative stress, and regulating the equilibrium of collagen remodeling[53]. They can potentially avoid the potential risks associated with direct stem cell transplantation. Stem cell-derived exosomes may be a future research trend.

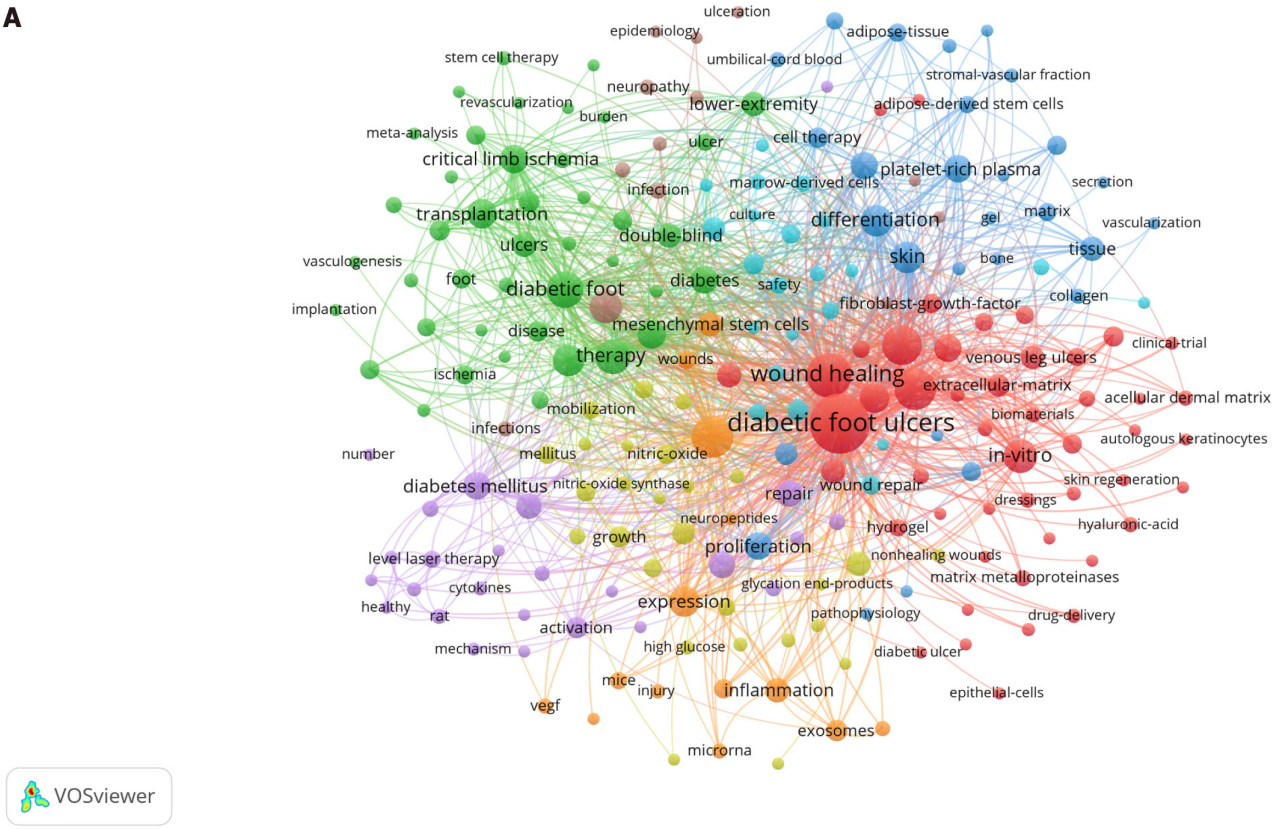
In chronic wounds, stem cell survival without scaffold support is short-lived. Multifunctional hydrogel wound dressings play a crucial role in the healing of skin wounds, as they can sustain stem cell viability for an extended period, provide moisture, and prevent electrolyte and fluid loss in DFUs[54]. Furthermore, photobiomodulation may also play an important role in stem cell applications. The combination of stem cells and photobiomodulation has shown the potential to accelerate the healing process of diabetic wounds[47].

Hotspots and frontiers

Keywords summarize the research topics and core content. Based on keyword co-occurrence analysis, it is possible to understand the distribution and development of various research hotspots in a specific field[55]. The keyword clustering ultimately identified eight possible research directions, including "diabetes mellitus," "expression," "chronic wounds," "adipose-derived stem cells," "diabetic foot," "diabetic wound healing," "double-blind," and "biological therapies." The most common keywords were "diabetic foot ulcers," "wound healing," "mesenchymal stem cells," "stem cells," and "angiogenesis." In addition, the most frequently mentioned keywords, included "repair," "skin," "venous leg ulcers," "efficacy," and "mechanisms." The aforementioned words indicate that the use of stem cells in DF has not only been examined in basic research studies but has also been investigated in clinical translation studies.

A growing body of research indicates that MSCs are capable of enhancing angiogenesis and epithelial remodeling, engaging in immune regulation, mitigating inflammation, and ultimately contributing to the facilitation of DFU repair. They have become an effective therapeutic approach for treating DF[56]. It is worth noting that controlled studies conducted using animal models indicated that combining stem cells with biostimulants (such as photobiomodulation) can reduce biofilm formation and expedite the healing of infected diabetic wounds[57]. Multiple randomized controlled trials have confirmed that stem cell therapy is a promising treatment for DF, as it can improve healing rates and reduce the amputation rate[58]. There is good clinical evidence promoting the clinical application and translation of stem cell therapy for DF. When selecting clinical stem cell types, considerations should be given to the availability and supply of stem cells, such as ease of acquisition, good manufacturing practices, and broad *in vitro* proliferative capacity[59]. Because of their convenient procurement, uncomplicated extraction methods, and documented safety profile, adipose-derived stem cells and other stem cell variants have become increasingly popular. Consequently, they have emerged as pivotal areas of concentration within research endeavors[60]. Beyond their regenerative characteristics and capacity to stimulate blood vessel development, stem cells sourced from adipose tissue exhibit a greater content when contrasted with bone

A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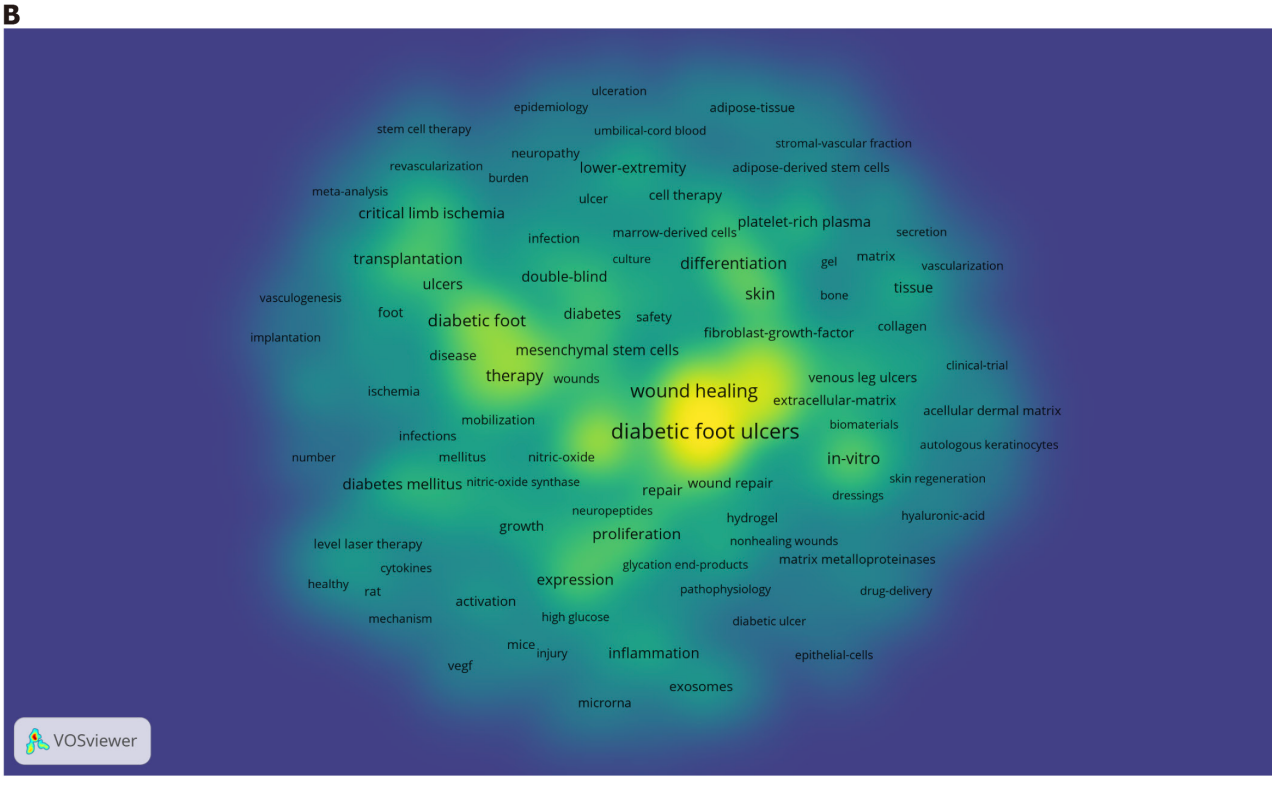
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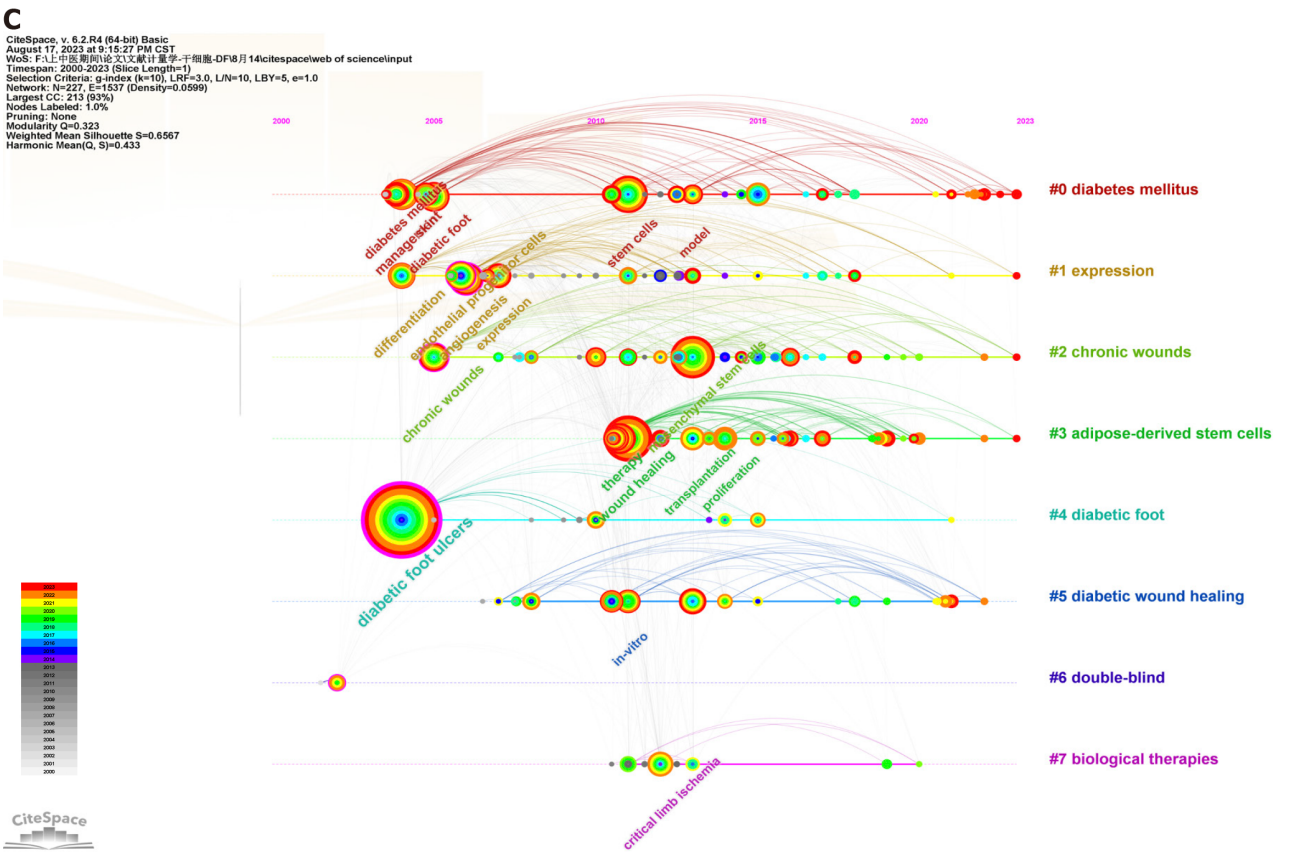

D **Top 10 keywords with the strongest citation bursts**

Keywords	Year	Strength	Begin	End	2000 - 2023
endothelial progenitor cells	2006	3.31	**2006**	2014	
mechanisms	2012	3.83	**2012**	2015	
repair	2015	5.15	**2015**	2018	
venous leg ulcers	2015	3.93	**2015**	2019	
efficacy	2019	3.86	**2019**	2021	
mesenchymal stem cells	2013	3.6	**2019**	2020	
skin	2005	4.15	**2020**	2020	
diabetic wound healing	2021	3.28	**2021**	2023	
proliferation	2014	3.64	**2022**	2023	
extracellular-matrix	2019	3.35	**2022**	2023	

DOI: 10.4252/wjsc.v16.i1.33

Figure 8 Analysis of keywords. A: Co-occurrence and clustering of keywords; B: Density map of keywords co-occurrence; C: Top 8 cluster timeline distributions; D: Top 17 cocited references with the most citation burstiness.

marrow aspirate-derived stem cells[59].

Through the utilization of bibliometric analysis, this study systematically presented the body of research concerning stem cell therapy in DF cases while also identifying focal points and burgeoning trends within this domain. Therefore, we have also depicted the potential mechanism diagram of stem cell research in the field of DF in Figure 9. Nevertheless, there are certain limitations associated with this study. First and foremost, the study's scope was confined to the WoSCC database for literature screening, potentially resulting in the omission of pertinent articles. This is due to the limitations of current bibliometric software, which makes it difficult to analyze multiple databases simultaneously. Additionally, this study utilized only a few tools, such as VOSviewer, CiteSpace, and R bibliometrics package, which may not fully explain the data. In future research, we intend to explore the use of other tools, such as artificial neural networks, to further analyze and interpret the data.

CONCLUSION

The bibliometric analysis indicated that research on stem cell therapy in DF has been rapidly progressing and holds great prospects for the future. The United States and China are scientific hubs for the research of stem cells in DF. In this field, Iran's Shahid Beheshti University Medical Sciences demonstrated the highest productivity, with Dr. Bayat from the same

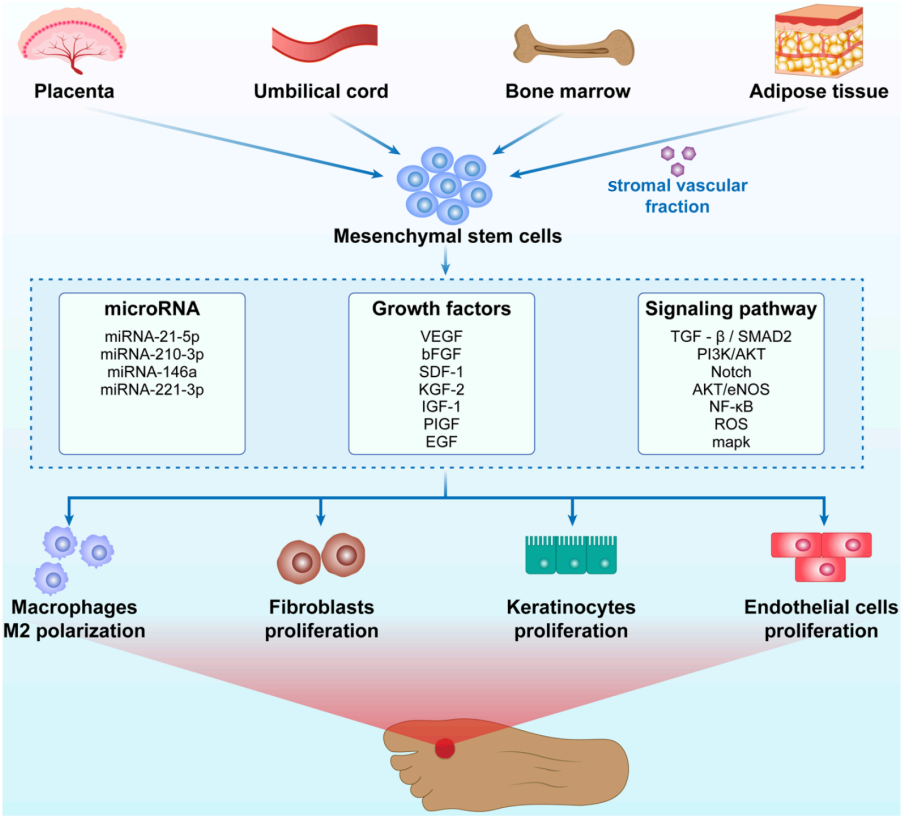

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Figure 9 Mechanism diagram of stem cell research in the field of diabetic foot. bFGF: Basic fibroblast growth factor; EGF: Epidermal growth factor; eNOS: Endothelial nitric oxide synthase; IGF: Insulin-like growth factor; KGF: Keratinocyte growth factor; NF-kB: Nuclear factor-kappa B; PI3K: Phosphatidylinositol 3-kinase; PIGF: Phosphorylated insulin-like growth factor; ROS: Reactive oxygen species; SDF: Stromal cell derived factor; TGF-β: Transforming growth factor-β; VEGF: Vascular endothelial growth factor.

university being an outstanding researcher in this domain. The priority topics revolved around dressings, extracellular vesicles, wound healing, and adipose stem cells. The results of these analyses will help researchers understand the current research status and provide hopeful directions for future studies. Future research will also focus on the clinical translation of stem cell therapies for DF.

ARTICLE HIGHLIGHTS

Research background

Stem cell therapy has shown great potential for treating diabetic foot (DF).

Research motivation

There is currently a lack of comprehensive research in this field.

Research objectives

The purpose of this study was to conduct a bibliometric analysis of studies on the use of stem cell therapy for DF over the past two decades, with the aim of depicting the current global research landscape, identifying the most influential research hotspots, and providing insights for future research directions.

Research methods

We searched the Web of Science Core Collection database for all relevant studies on the use of stem cell therapy in DF. Bibliometric analysis was carried out using CiteSpace, VOSviewer, and R (4.3.1) to identify the most notable studies.

Research results

A search was conducted to identify publications related to the use of stem cells for DF treatment. A total of 542 articles published from 2000 to 2023 were identified. The United States had published the most papers on this subject. In this field, Iran's Shahid Beheshti University Medical Sciences demonstrated the highest productivity. Furthermore, Dr. Bayat from the same university has been an outstanding researcher in this field. *Stem Cell Research & Therapy* was the journal

with the highest number of publications in this field. The main keywords were "diabetic foot ulcers," "wound healing," and "angiogenesis."

Research conclusions

This study systematically illustrated the advances in the use of stem cell therapy to treat DF over the past 23 years. Current research findings suggested that the hotspots in this field included stem cell dressings, exosomes, wound healing, and adipose-derived stem cells.

Research perspectives

Future research should also focus on the clinical translation of stem cell therapies for DF.

FOOTNOTES

Author contributions: Liu GB, Yang X, and Fan WJ proposed the research design; Shi HS and Yuan X conducted the literature search, publication screening, and data extraction; Shi HS carried out the data analysis; Wu FF and Li XY conducted manuscript revisions; Hu XM made important contributions to the editing and illustration of this manuscript; The authors collectively contributed to and endorsed the final version of the article.

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