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REVIEW ARTICLE

Regenerative medicine in surgery: stem cells and exosome applications

Medicina regenerativa en cirugía: aplicaciones de células madre y exosomas

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Abstract

This review outlines the role of regenerative medicine in surgical applications, focusing on stem cells and exosomes. Among the most important features of stem cells are their unique ability to differentiate into various cell types, which make them stand out in regeneration and repair. Recent advances highlight the effectiveness of not only these cells themselves but also the exosomes, the nano-sized extracellular vesicles they produce, in this regeneration. On the other hand, exosomes assure additional advantages concerning low immunogenicity and high bioavailability but have raised problems in standardization and safety. In fact, the introduction of these stem cell and exosome technologies is changing the management of surgical pathologies and offering hope for diseases hitherto considered incurable. In this review, the use of stem cell therapies in various surgical diseases is categorized and examined, their clinical importance is emphasized, and the deficiencies in research studies are indicated. Therefore, the results are promising for treatments, but more standardized protocols and expanded research on long-term safety and effectiveness are needed.

Keywords: *Regenerative medicine. Stem cell. Exosomes. Clinical applications. Surgery.*

Resumen

Esta revisión describe el papel de la medicina regenerativa en aplicaciones quirúrgicas, enfocándose en células madre y exosomas. Entre las características más importantes de las células madre destaca su capacidad única de diferenciarse en diversos tipos celulares, lo que las hace sobresalir en regeneración y reparación. Los avances recientes resaltan la efectividad no solo de estas células en sí mismas, sino también de los exosomas (las vesículas extracelulares de tamaño nanométrico que producen) en estos procesos de regeneración. Por otro lado, los exosomas ofrecen ventajas adicionales relacionadas con baja inmunogenicidad y alta biodisponibilidad, aunque han planteado problemas en cuanto a estandarización y seguridad. De hecho, la introducción de estas tecnologías de células madre y exosomas está transformando el manejo de patologías quirúrgicas y

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Date of reception: 25-11-2024
Date of acceptance: 14-12-2024
DOI: 10.24875/CIRU.24000620

Cir Cir. 2025;93(1):86-104
Contents available at PubMed
www.cirugiaycirujanos.com

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ofreciendo esperanza para enfermedades hasta ahora consideradas incurables. En esta revisión se categoriza y examina el uso de terapias con células madre en diversas enfermedades quirúrgicas, se enfatiza su importancia clínica y se señalan las deficiencias en los estudios de investigación. Por lo tanto, los resultados son prometedores para los tratamientos, pero se necesitan protocolos más estandarizados y una investigación ampliada sobre la seguridad y efectividad a largo plazo.

Palabras clave: *Medicina regenerativa. Células madre. Exosomas. Aplicaciones clínicas. Cirugía.*

Introduction

Stem cells, which have the ability to differentiate into various cell types, play a crucial role in regenerative medicine, particularly in surgical applications. These cells can be classified according to their differentiation potential, ranging from totipotent, which can form all tissue types, to unipotent, which are limited to a single cell type[1]. Unique properties of stem cells, such as differentiation, self-renewal, and survival, make them invaluable for tissue regeneration and repair. In recent years, the importance of stem cell-derived exosomes in intercellular interactions has become more prominent, and their preference for use has also increased[1,2]. Exosomes carry proteins, lipids, and nucleic acids, making them potent tools for diagnostics and therapeutic interventions. The use of exosomes in clinical settings offers advantages such as reduced immunogenicity and improved bioavailability[2,3]. However, the lack of optimizations of fixed and worked protocols still brings some difficulties in terms of security. The integration of stem cell and exosome technologies is paving the way for innovative treatments in surgery, offering hope for patients with previously untreatable conditions[1-3].

This review aims to thoroughly analyze and examine the studies related to stem cell research conducted in various surgical diseases and within the broader context of surgical and medical sciences. By critically evaluating the methodologies and outcomes of these studies, we intend to provide a detailed understanding of their significance and potential clinical applications. The review seeks to shed light on the advancements in stem cell therapies specific to surgical conditions while also considering their implications for general medical practice. In addition, this analysis will identify existing gaps in the research and suggest directions for future studies, ultimately contributing to the advancement of stem cell-based treatments in both surgical and medical fields.

Stem cell system

Stem cells have the ability to transform into many cell types according to their source and differentiation potential. These cells have great potential for future therapeutic use in the fields of tissue regeneration and repair. Just like every cell has specific characteristics, stem cells need to have certain features to be stem cells. Among these features are self-renewal and differentiation[1]. Self-renewal refers to a cell's capacity to divide and create other cells with the same characteristics, while differentiation refers to the ability to create other cell types that perform different biological functions[2]. If we classify stem cells, we can divide them into four groups according to their developmental potential: totipotent, pluripotent, multipotent, and unipotent (Fig. 1). In this section, we will discuss the classification of stem cells. First, totipotent stem cells are differentiated cells capable of forming all tissues of the embryo and extra-embryonic tissues. These cells are obtained from the fertilized oocyte and the first blastomeres. They are also present in the early stages of the embryo and have a very broad differentiation potential[4]. Pluripotent stem cells are also having the ability to differentiate into the body's three germ layers: ectoderm, endoderm, and mesoderm. Pluripotent stem cells play a key role in disease models, drug release systems, cell therapy, and provide an important experimental platform[5]. There are several methods for the differentiation of pluripotent stem cells, including chemical activin A and the growth factor BMP-4[6]. Unlike totipotent cells, they cannot produce cells from extra-embryonic tissues. Multipotent stem cells, the most distinctive feature, are their ability to differentiate into a specific tissue. They are generally used for the regeneration of various body tissues. These stem cells have more limited differentiation abilities compared to other stem cells[6]. Finally, unipotent stem cells are groups of stem cells with limited capabilities, similar to multipotent stem cells. It can only differentiate into the cell type in their niche. For example, muscle stem cells can only lead to mature muscle cells[7]. Characterization of stem cells various factors affects stem cell cultures. These factors include cell density, density of feeder cells, growth factors, microbial contamination, and others. Techniques such as flow cytometry, karyotyping, fluorescence *in situ* hybridization, single-nucleotide polymorphism, immunocytochemistry, and western

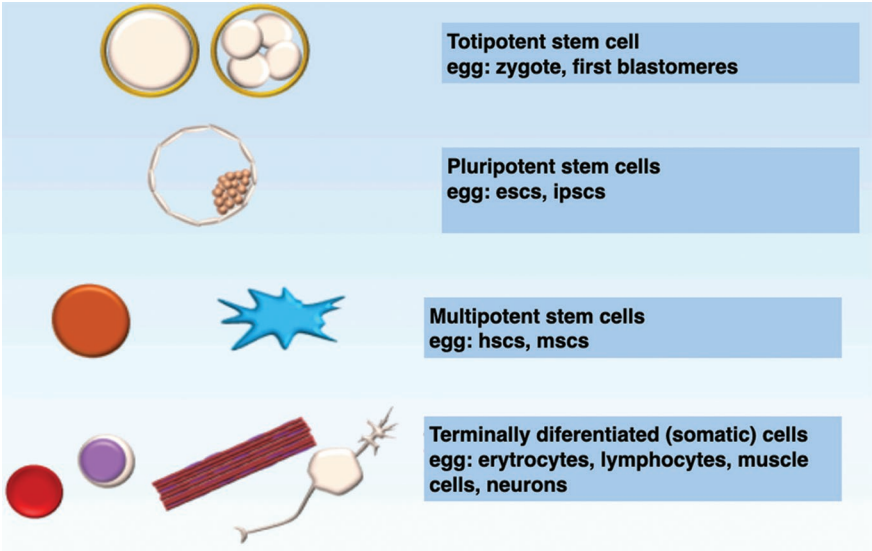

Figure 1. *Stem cell types.*

blot are preferred for the genetic characterization and analysis of stem cells. Some types of stem cells, especially pluripotent stem cells, are more prone to genetic instability and chromosomal changes and should be frequently observed. Stem cells express unique and specific combinations of transcription factors, cell surface proteins, and cytoplasmic proteins[8].

Exosomes technology: how does it work in clinical?

Exosomes are nano-sized extracellular vesicles naturally secreted by eukaryotic and prokaryotic cells, with diameters ranging from 30 to 150 nm and attracting intense interest in the field of biotechnology as they participate in intercellular communication[9]. Although the biogenesis mechanism of exosomes is controversial, in general, they are synthesized in early and late endosome stages of the cell and released outside the cell when these endosomes fuse with the plasma membrane[10]. Their biological functions and characteristics differ according to the cell types from which they are released and they carry a large amount of information due to their proteins, lipids, and nucleic acids. For this reason, they are considered as potential biomarkers to be used in the diagnosis and treatment of diseases. Animal-derived exosomes (ADEs) from mammals and plant exosomes, which have been increasingly studied in recent years, are the two main categories of naturally derived exosomes[11]. ADEs secreted by macrophages, mesenchymal stem cells (MSCs), dendritic cells, endothelial cells, and tumor cells play key roles in a variety of vital processes, including cell growth, proliferation, and differentiation, intercellular communication, angiogenesis, inflammation, immune responses, and tumor formation and development[11-13]. A study found that plant-derived exosome-like nanoparticles (PDENs) for the 1st time in carrot cells in 1967[14] and over time, studies have shown that nano-sized EVs obtained from plant cells have structures similar to mammalian exosomes[15,16]. Therefore, these EVs were called "plant-derived exosome-like nanoparticles"[17]. Since their discovery, PDENs have been studied for their pharmacological effects in various areas such as tumor, liver diseases, and skin wound healing[18]. Plant exosomes have a larger nanoparticle size and a complex content of small RNAs, proteins, lipids and other metabolites like mammalian exosomes[19]. The differences between the two forms of exosomes are detailed in table 1[19,20-28].

Although ACEs offer various benefits as therapeutic targets, diagnostic indicators, and drug delivery reporters, their clinical translation remains elucidated due to limitations such as possible toxicity, tissue-specific targeting issues, and low efficiency. PDENs have the advantages of not being detected by the immune system and having better bioavailability. They can also target specific organs, have fewer side effects, and dissolve faster[19,29]. Its use in the surgical field attracts attention in a wide range of areas, especially from wound studies to IVF, central nervous system applications to orthopedics[10,29].

Characterization techniques have been improved to the morphological and physicochemical properties of exosomes, as well as to distinguish and differentiate them from other extracellular vesicle subsets. There are three commonly used characterization methods for observing the morphology of exosomes: transmission electron microscopy, scanning electron microscopy, and atomic force microscopy[30]. While the advantage of these methods is that they require fewer samples, the disadvantage is that the preparation methods of the sample may cause shape changes. The most frequently used methods to determine the size distribution of exosomes are dynamic light scattering and nanoparticle tracking analysis (NTA). Fast and real-time observation makes the NTA method more advantageous. Western-blot and enzyme-linked immunosorbent assay methods are preferred for the determination of surface proteins of exosomes, while flow cytometry is used for exosomal biomarkers, allowing qualitative and quantitative analysis of exosomes. Flow cytometry offers high throughput and multi-channel analysis with few samples. The three approaches have the drawback of being arduous, complicated, and time-consuming[31].

Exosome biogenesis involves endocytosis, early endosome formation, multivesicular body (MVB) formation, and ultimately exosome release[32,33]. Exosome biogenesis begins with the accumulation of material taken up from the plasma membrane through endocytosis in the early endosomes[34]. At this stage, endocytic vesicles are detached from the plasma membrane and taken into the cell. Early endosomes are the first stop for molecules taken up from the cell surface for sorting and processing of materials[35]. At this stage, biomolecules, lipids, or proteins that are present on the cell surface and come from outside the cell are taken up by endocytosis and transported to early endosomes. Early endosomes mature into late endosomes. In this step, MVBs are formed[36]. MVBs are intraluminal vesicles (ILVs) containing large endosomal structures. ILVs are the precursors of exosomes and are formed by budding from the inner membrane of MVBs[33]. This process is critical for intracellular signaling and protein transport. MVB formation is an important step in intracellular transport and material processing. The formation of ILVs is regulated by the endosomal sorting complex required for transport (ESCRT) mechanism and ESCRT independent mechanisms[36]. ESCRT Mechanism: ESCRT-0 Complex (Recognizes and recruits ubiquitinated forms of surface proteins. This complex is the first step in the selective transport of proteins), ESCRT-I, ESCRT-II Complexes (Locates on the inner membrane of the MVB and creates a bend. These complexes play a key role in the budding step of ILVs), and ESCRT-III Complex (Completes membrane bending and ensures budding of ILVs into the MVB. ESCRT-III regulates the final closure of the membrane and the formation of ILVs)[37]. VPS4 ATPase: Ensures the separation of ESCRT complexes from the membrane and is effective in terminating the process[38]. ESCRT Independent Mechanisms: Tetraspanins, Crohn's disease (CD)63, CD81, and similar tetraspanin proteins play an critical role in ILV formation[39]. Tetraspanins promote ILV budding by forming membrane microdomains. Membrane microdomains are formed by local accumulation of lipids such as lipid raft and ceramides. These microdomains ensure membrane budding and ILV formation[40]. MVBs are transported intracellularly along cytoskeletal elements (microtubules and actin filaments). MVBs can follow two main pathways; lysosomal degradation and exosome secretion by fusion to plasma membrane MVBs fuse with lysosomes and digest the material inside them. The lysosomal degradation pathway is effective in recycling proteins and clearing intracellular waste. In the other pathway, MVBs go to the plasma membrane and fuse there, releasing the ILVs inside them as exosomes. This process allows the active material to be transported out of the cell and the exosomes to perform their biological functions[33]. Exosomes perform various biological functions after being released out of the cell (Fig. 2).

Transfer to humans: good manufacturing practices (GMP) conditions

They have a critical role as a safe and cell-free therapy in many immunological and degenerative diseases. Nonetheless, the isolation and translation to clinical applications might be restricted by insufficient large-scale manufacturing techonologies. There are many details and practices that need to be taken into consideration under GMP standards before applying it to the clinical trials[41-43]. It is important to evaluate cytotoxicity, hemolysis, and DNA damage of exosomes at the cellular levels and to test their effects at the animal levels including tissues, organs and their biodistribution[42]. Furthermore, it is crucial to test endotoxin levels, bacterial sterility, and mycoplasma detection and stored at (–80)°C until use[43].

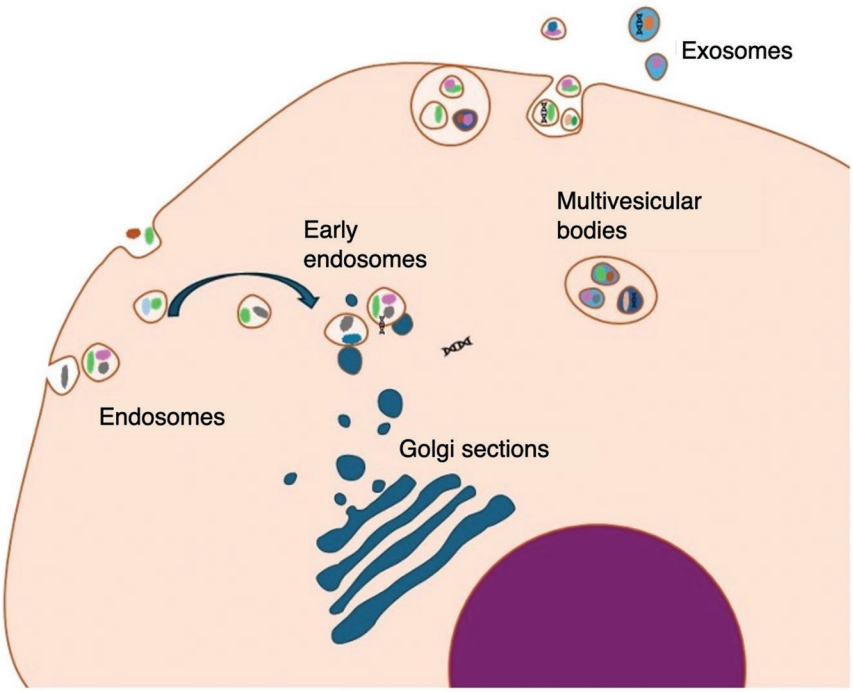

Figure 2. *Exosomes biogenesis.*

It must be applied in the methods including cell culture, exosome extraction, purification, and characterization for physical structure and bioactivity function under GMP condition. The ultracentrifugation is most critical in isolation of exosomes among these methods in the clinical trials. Exosome applications in clinical trials also have some challenges including non-homogenity, fast clearance from the body, and long-term preservation stability. To achieve clinical success, therapeutic doses and routes of administration need to be standardized[41].

Applying regenerative medicine in surgery

General surgery field

Liver failure resulting from portal vein thrombosis is a challenging and frequently encountered condition with difficult management and treatment. A systematic review by Stutchfield et al. Indicated that after the injection of stem cells isolated from peripheral blood mobilized with granulocyte colony stimulating factor or from the iliac crest bone marrow, induced pluripotent stem cells (iPSC), bone marrow MSCs (BMMSC), and adult stem cells (SSC) into the portal vein or hepatic artery, liver regeneration was observed[44]. Although it is believed that embryonic stem cells would also accelerate hepatic regeneration, studies on embryonic stem cells remain insufficient due to ethical concerns[44]. In cases of lymphedema following mastectomy and axillary dissection surgery for breast cancer diagnosis, a decrease in lymphedema and an increase in lymphangiogenesis were demonstrated following combined therapy with traditional decongestive treatment and intramuscular injection of adipose stem cells (ASC) containing MSC[45]. It is difficult to achieve positive results in surgery for complex perianal fistulas (CD or cryptoglandular type) in the short and long term. However, recent metaanalyses and clinical studies indicate that MSCs transplantation may be an effective and sustainable therapeutic method for the treatment of complex perianal fistulas in both short and long term[46-49]. For the treatment of pelvic organ prolapse, the use of autologous muscle-derived cells, fibroblasts, or MSCs added to bioresorbable, fragmentable, and potentially growth-promoting scaffolds is considered an alternative to traditional surgical reconstructive methods[50]. Proctitis is a complication frequently encountered as a result of radiation therapy in the treatment of various pelvic malignancies (rectal, gynecological, and urological cancers). The studies indicate that MSCs therapy can be considered as an

alternative treatment method in the management of this complication, in addition to treatment methods such as argon plasma coagulation, laser, radiofrequency ablation, and cryoablation. A study by Kim et al. on rats showed that stem cell application reduced fibrosis and increased new cell proliferation[51,52].

Gastroenterology and hepatology field

Liver is an important organ of human immune system. It is known that many liver diseases occur due to immune homeostasis imbalance[53]. MSCs are non-hematopoietic stem cell with self-renewal and differentiation capacity[53]. Studies have shown that they can re-establish the immune hemostasis, inhibit the activation of innate and adaptive immune cells, promote tissue regeneration, alleviate liver damage, and increase liver function[53-55]. MSCs exhibit these function mainly through their excreted extracellular vesicles (MSC-EV) and exosomes which are important component of these paracrine secretion. However, due to the breadth of the subject, this review will mainly focus on the clinical applications of MSCs. Different cell types with different delivery routes are used either allogenic or autologous. BMSCs were commonly used, followed by umbilical cord-derived MSCs and adipose-derived MSCs. Although there are reports with hepatic arterial and intrahepatic injection, peripheral intravenous line is the most commonly used delivery route[53]. *In vivo* studies demonstrated beneficial effect of MSC on certain liver diseases. It has been suggested that their beneficial effect is elicited through promoting anti-inflammatory M2 macrophage polarization and increasing IL-10 secretion in non-alcoholic steatohepatitis; increasing Treg/TH17 proportion in autoimmune hepatitis (AIH); activating of NLRP3 inflammasome and downregulating the Interleukin 6 (IL-6) signal pathway in acute liver failure, downregulating the tumor necrosis factor α (TNF-α) and Interleukin-1 β (IL1β) expression in liver fibrosis, downregulating CD154 expression, and enhancing the shift of TH17 toward Treg in ischemic liver disease. Focusing on different liver disease, autologous BMMSC transplantation revealed inconsistent results in non-viral etiology. A single-blinded controlled clinical trial performed with 27 decompensated cirrhotic patient including hepatitis C virus (HCV), hepatitis B virus (HBV), and AIH patients could not demonstrate any beneficial effect[54,55]. Another single-center randomized controlled study evaluated 9 grade 2-3 cirrhotic patients. They received 5 IV infusions of 1.0 × 10^6 cells/kg, BM-MSC twice a week for 2 week and another dose at 3rd week. Although MSC leads to significant improvement in Child-Pugh and model for end-stage liver disease (MELD) scores, 90-day survival rates were similar between the MSC and placebo groups[56]. Similarly, a randomized controlled clinical trial enrolling alcoholic cirrhotic patient reported no impact of BM-MSC on liver histology[57] Despite these disappointing results, studies with viral hepatitis and AIH seem more promising. In a prospective controlled randomized study, enrolling 110 HBV-related acute on chronic liver failure patient BM-MSC treatment decreased the severe infection rate and increased survival rate at the 24-week after transplantation[58]. Furthermore, in long-term follow-up, peripheral allogenic umbilical cord-derived MSCs (UC-MSC) therapy markedly improved liver function and reduced the volume of ascites in patients with HBV-related decompensated liver cirrhosis up to 75 month[59,60]. In another study enrolled with HBV infected cirrhotic patient, BM-MSC was given through hepatic artery injection and improvement of liver function was observed through downregulating TH17 and promoting Treg development, at 24 week follow-up[61]. Similar encouraging results are reported also in HCV infection-related liver disease. Peripheral vein infusion of autologous BM-MSC in HCV-related cirrhosis demonstrated improvement in liver function in a prospective randomized phase II clinical trial[62,63]. Regarding AIH, in fact, there are considerable amount of *in vitro* and *in vivo* studies related to the effect of stem cell on AIH[64]. However, clinical implications of these seem weak. The effect of allogenic MSC was evaluated in one study. The trail enrolled 26 patients with cirrhosis related to AIH. Patient received mainly UC- MSC through peripheral line. Although mean alanine transaminase level decrease was not significant at 6 month, and 2 years follow-ups, MELD score improvement was significant at 6 months[65]. There are also reports that allogenic BM-MSC safely improved quality of life and liver function in suboptimal ursodeoxycholic acid (UDCA) resistant primary biliary cirrhosis[66].

Another area where stem cell-based therapies have been suggested as promising source to improve disease state is inflammatory bowel diseases (IBD). Although the etiology of IBD has not been highlighted; completely, yet dysregulation of innate and adaptive immune pathways contributes to the development of aberrant inflammation[67]. Theoretically, stem cell transplantation can reset the dysregulated immune system, re-establish the immune homeostasis, and repair and

supplement intestinal epithelial cells leading to remission. To date, there have been numerous clinical studies on stem cell transplantation for the treatment of IBD utilizing different MSC origin. Nevertheless, most of these studies have confirmed the efficacy of MSCs in treating CD with desirable safety and seems highly promising approach for especially fistulizing perianal CD. However MSC, still less effective in luminal disease[68]. The application line of MSC has also been shown to be effective in achieving remission. For IBD treatment, intravenous, intraperitoneal, or local application is possible. Compared to peripheral or intraperitoneal injection, local applications have been found to be more effective in PFCD[67,68].

The clinical studies demonstrated encouraging results with adipose tissue-derived stem cell and darvadstrocel/Cx601 (Takeda) (allogeneic adipose-derived stem cells), which has already entered clinical treatment. A phase III multicenter randomized, double-blind placebo-controlled trail studied the efficacy of allogeneic ASC transplantation on treatment refractory CD with draining complex perianal fistula. The study comprised 212 patients who were assigned into treatment and placebo groups and received a single injection of Cx601 cells or placebo in addition with standard treatment. The study evaluated magnetic resonance imaging (MRI) proofed closure of all openings, and absence of draining fistulas termed as combined remission and clinical remission respectively at short term. At 24-week, a significantly greater proportion of patients treated with Cx601 versus placebo, achieved combined remission (51% vs. 36%, $p = 0.021$). Moreover, significantly less adverse event was observed in ASCT-(cx-601) treated group compared to placebo (17% vs. 29%, respectively). Same authors assessed the durable effect of Cx-601 in a second trail and observed that the efficacy and safety maintained for up to 52 weeks after treatment. A significant proportion of the treatment group compared to control group had combined remission at 52 weeks after treatment (56.3% vs. 38.6%, respectively)[67]. The authors concluded that Cx601 is an effective and safe treatment for patients with refractory, complex, and active perianal fistulas both in short and long term. Consistent with this result Barnhoorn et al. reported even longer durable clinical remission, fistula closure up to 4 years in previously BM-MSC transplanted treatment resistant perianal CD patients[68]. MSCs have also been reported as an effective and safe treatment for intractable intraluminal IBD. A randomized controlled trail enrolled 82 patients with refractory intracavitary CD. Forty-one of the patients were assigned to receive 1×10^6 UC MSCs/kg once a week. They injected a total of four peripheral intravenous infusions and assessed after 12 months of treatment. The clinical disease activity index decreased by 62.5 ± 23.2 in the intervention group compared to 23.6 ± 12.4 in the control group[69]. The studies on ulcerative colitis and MSCs therapy are also progressing. As in CD, local injection with colonoscope of MSCs has shown promising results also in ulcerative colitis[70]. In contrast, intravenous injection warrants further investigation. A single-center randomized controlled trail evaluated the efficacy and safety of Human Umbilical Cord MSCs in moderately active ulcerative colitis patient. The study included 70 patients who were randomly and almost equally assigned into treatment and control group (34 vs. 36 patients, respectively). The patient received MSC twice through peripheral vein. In addition to stable baseline 5ASA therapy, a third dose was administered through interventional catheterization into the superior mesenteric artery at a 7-day interval. The median Mayo score improved in the intervention group at 3 months after cell therapy and reached its lowest level at 6 months. It, then, continued or fluctuated slightly during the 24-month follow-up period. The clinical response rate was 85.3% in the intervention group and 16.7% in the control group. Interestingly, this study did not show any changes in mean plasma cytokine levels, including TNF-α, IL-6, and Interferon-γ. It failed to show any changes, which were shown to be significantly attenuated in the luminal Crohn's disease cohort treated with MSC culture in combination with AZA[71].

Pediatric surgery and pediatric urology field

Pediatric surgery is relatively the most recently established discipline within the field of medicine. Consequently, it is expected that pediatric surgery lags significantly behind other medical fields in terms of advancements in stem cell research. Indeed, while branches such as internal medicine and orthopedics lead the way in stem cell applications, discussions about the implementation of stem cell techniques in pediatric surgery have only just begun to emerge in recent medical conferences. Recently, the application of stem cell therapy in adults with liver cirrhosis has yielded promising results[72,73]. A study involving 12 children with BA who received stem cell transplantation showed mixed outcomes. Follow-up indicated decreased cholangitis and improved liver function in some patients, though five children succumbed to ongoing cirrhosis[74].

In 2011, Sharma et al. reported on how bone marrow cell transplants helped 11 patients with biliary atresia that had previously undergone the Kasai operation[75]. They found that patients who received stem cell therapy along with the Kasai operation showed improvements in their health compared to those who only had the Kasai procedure. Another study conducted by Nguyen et al., from 2017 to 2019 investigated the use of an autologous bone marrow cells (BMMNC) to treat liver cirrhosis caused by biliary atresia in children who had already undergone the Kasai operation[76]. The research involved 19 children, where bone marrow was collected and specific cells were separated. These cells were then infused into common hepatic artery twice over 6-month intervals. The study showed no overall side effects. Although one child passed away during the study period, the remaining 18 children demonstrated improvements in liver function, as evidenced by lower pediatric end-stage liver disease scores and improved biochemical markers. The research findings indicated that the use of BMMNC infusion could be a beneficial approach to improving liver function in children with biliary atresia. In addition, Nguyen et al. reported a significant decrease in the incidence of cholangitis following the administration of BMMNC. Before receiving BMMNC, all patients had experienced one or more episodes of cholangitis, but none of them experienced cholangitis after the treatment. Recently, some clinical trials on the application of stem cells in biliary atresia patients have been initiated. We also have an ongoing study where we are applying stem cell therapy in biliary atresia (NCT06564740)[77]. The protocol and infusion method for stem cell application in patients with biliary atresia are illustrated in figure. 3.

Stem cell therapy is also crucial in treating necrotizing enterocolitis (NEC)[78]. The recent emergence of stem cell therapy, which exhibits multi-directional differentiation capacity, self-renewing potential, and good abundance, has positively affected intestinal barrier function[79,80]. However, it can also lead to increased apoptosis in the presence of inflammatory reactions. These beneficial results have been studied recently for treating NEC diseases[78-80]. Yet, it is unknown whether the benefits of stem cells in NEC are preventive or protective, which might also determine the optimal time for delivery[81]. Experimental models simulating the condition of NEC suggest that stem cell therapy is effective. A full-term infant with NEC was operated on at Day 22 after birth and received intravenous UC-MSCs postoperatively. The circulation perfusion in the mesenteric area was remarkably improved. Stem cells in this case improved the prognosis of NEC and prevented short bowel in a critical infant[82].

Undescended testis is another diagnosis in pediatric surgery where there are significant future expectations for stem cell applications. It has been reported that BM-MSCs may differentiate into various types of cells at the site (and even locally fused with adjacent tissue), or act as chemoattractants to recruit local endogenous stem/progenitor cells to participate in compensating for damaged tissues[83-85]. One such study was conducted by Alhefnawy et al., using a single-dose intratesticular injection of MSCs in adult patients with non-obstructive azoospermia (NOA)[86]. In their study, out of 87 patients, 18 (20.7%) showed the presence of sperm in their semen at various times. As far as we know, there has not yet been published any article regarding stem cell applications in children for undescended testis. Undescended testis, a common condition in children, is an important cause of NOA. From this viewpoint, stem cell therapy may provide a promising option for patients undergoing surgery for undescended testis[87].

Musculoskeletal (MSK) system diseases

MSK disorders are a wide range of diseases and conditions that affect the muscles, bones, joints, and associated connective tissues. MSK disorders related disabilities can lead to loss of productivity, work absenteeism, early retirement, and increased healthcare costs[88]. MSCs, obtained from various autologous and allogenic sources, have promising effects on MSK pathologies, attributed to their ability for self-proliferation and multipotency. All clinical studies conducted today are based on the ability of mSCs to undergo osteochondrogenic differentiation, their ability to support stem cells in the bone marrow, their ability to suppress the immune system, and their trophic effects[89].

In MSK practice, MSCs are mostly used in cartilage lesions. MSCs create normal articular cartilage or mature hyaline-like tissue even in full-thickness cartilage defects through paracrine cell signaling[90-94]. In a study, autologous BMMSC found to be as efficient as autologous chondrocyte implantation[95]. In another study of patients with knee osteoarthritis divided into two groups, high tibial osteotomy was treated with autologous MSC injection or arthroscopy. It was observed that the chondral defects of the MSC injected group closed better, but no clinical difference was found[96]. Centeno et al.[97] examined the patients treated by high-dose or low-dose bone marrow aspirate concentrate (BMAC) injections.

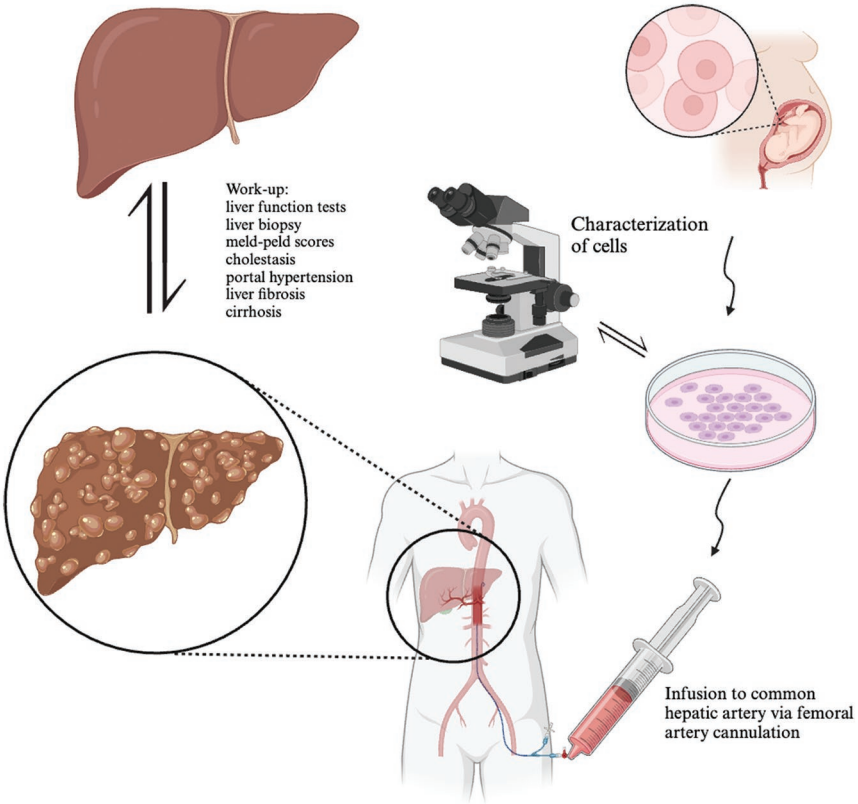

Figure 3. *Stem cell application model for biliary atresia patients (infusion to common hepatic artery through femoral arterial cannulation).*

In the evaluations made in terms of pain and functionality, positive and significant improvements were observed in both. Another study examined the efficacy of BMAC injections in knee and hip osteoarthritis (OA). Approximately 13 months after the injection, improvements in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores were seen[98]. In addition, long-term improvements in WOMAC scores have been reported in OA patients treated with platelet-rich plasma injections[99].

The inner 1/3 of the meniscus is a vascular. This area has no healing potential. Izuta et al.[100] reported avascular zone repair using mesenchymal tissue in the fibrin matrix in animal experiments of meniscal tears adipose tissue originated mesenchymal cells differentiate to menischal cells. Pak et al.[101] reported an increasing improvement in MRI of patients with Grade 2 meniscus tear after treated with autologous adipocyte-derived mesenchymal cells injection 3, 6, 12, and 18 months after the procedure. There is also a study suggesting that allogeneic MSCs have healing potential meniscus volume in some patients[102].

After the osteogenic potential of MSCs was demonstrated *in vitro*, studies showing their osteoblastic activities and contributions to bone formation *in vivo* were published[103-106]. Hidaka et al.[107] reported in a study that there was better union and fusion in the vertebral fracture area with the application of bone marrow cells.

Osteonecrosis may occur due to different causes or due to systemic steroid use. Abnormal proliferation of fat cells, fat accumulation in osteocytes, causes high intraosseous blood pressure and blood circulation disorders. This leads to osteonecrosis of the femoral head[108]. Treatment of osteonecrosis by MSC transplantation depends on the osteogenic effect of the transplanted cells on the femoral head[109,110].

MSC applications are also very promising in osteogenesis imperfecta by increasing type-1 collagen production, osteoblastic differentiation, and bone mineral density[111].

There are studies showing that MSC has effects on tendon and disc degeneration[112-119]. Awad et al.[112] have shown that MSCs promote healing in tendinopathy of rabbits. Chong et al.[113] showed that MSCs are

effective in the early phase of tendon healing on the rabbit Achilles tendinopathy. Hernigou et al.[114] revealed by ultrasound and MRI that all 45 patients who underwent MSC injection during arthroscopic rotator cuff repair recovered within 6 months, and 67% of the 45 patients (30 patients) who did not undergo MSC recovered within 6 months, and they reported that the rate of rerupture decreased over time. Selek et al.[115] reported that MSC application increased tendon strength due to antiapoptotic effects. The cell-based therapies have entered the literature as a highly suitable treatment strategy for patients with intermediate stages of intervertebral disc degeneration. The clinical studies have shown the potential impact of allogeneic BMSCs in terms of safety and feasibility[116,117]. Regression of vacuum phenomenon in MRIs of patients with intervertebral disc degeneration 2 years after MSC therapy has been reported[118]. In a pilot study, autologous BM-MSCs were incorporated in the nucleus pulposus and reported an increase in disc height and water content of NP, improvement in LBP, and disability at 12 months of injection[119].

Effect of exercise on stem cells

It is a widely accepted view that regular exercise has a regenerative and protective effect on the human body. This includes cardiac regeneration, neural regeneration, bone development, and muscle regeneration[120,121]. Exercise training is considered safe, simple and has extensive health effects[122]. In a study published in 2024, Takegaki et al.[123] discovered that the intramuscular injection of MSCs activated the transcription of insulin-like growth factor 1 and muscle protein synthesis 72 h after three bouts of resistance exercise. In addition, the injection of MSCs was found to increase protein ubiquitination, suggesting that basal muscle protein turnover may be augmented following consecutive resistance exercise. Guerci et al.[124] showed that serum response factor, a ubiquitous transcription factor expressed in muscle, can regulate the synthesis of IL-6, an event necessary for satellite cell proliferation in response to mechanical exercise in rodents. Taken together, these studies suggest an important role for the paracrine factor milieu in satellite cell activation after exercise. Finally, in a review by Song (2022)[121], current evidence suggests that mechanical loading is beneficial for better callus properties and faster bone regeneration. The clinical studies in elderly fracture patients have shown improved healing and MSK function[125].

Neurosurgery field

The stem cell studies in the field of neurosurgery also include the principles of tissue engineering. Peripheral and central neuroengineering include stem cell and exosome transport systems, conduit scaffolds, and graft discs[126-130]. These studies face various difficulties in translating from the clinic to practice. The studies on nerve damage with exosomes, compared to stem cells, may be more efficient in terms of ethical issues and controllability of toxicity, with lower immunogenic, teratogenic, and tumorigenic capacity. It is hopeful that nanomedical practitioners can transform the laboratory and clinical studies conducted so far into more practical applications in the future[129]. In particular, in traumatic brain injury and some ischemia studies, exosome and stem cell therapies target neuroinflammation by preventing cell death. Gao and colleagues have shown that exosomes from reprogrammed neuronal stem cells used in stroke stimulate both neuroinflammation and neurogenesis[131]. In another study, it was discovered that neuronal stem cell exosomes added to the medium with stress with H_2O_2 increased cell survival[132]. It has been demonstrated that exosomal miR-146a-5p derived from umbilical cord MSC can improve the activation and neuroinflammation of microglia cells *in vitro* and *in vivo*[133].

In a clinical study published in 2024, four cerebral palsy patients who were unable to stand or walk without external support were administered UC-MSCs intrathecally, intravenously, and intramuscularly at 1×10^6/kg 6 times allogeneically and measured using the Modified Ashworth Scale (MAS). A significant change was seen in MAS measurements on both sides, but no significant change was found in cognitive functions[134]. Especially in peripheral nerve injuries, combination therapies that include tissue-engineered neural conduits, scaffolds, stem cells, or exosomes are among the most current areas of nerve tissue engineering. In complete nerve transection injuries, compared to stem cell/exosome applications alone, stem cell/exosome applications carried as combination therapy in the scaffold significantly increase nerve terminal integration and neural stem cell differentiation efficiency[127,135].

In a peripheral nerve injury study conducted at the clinic and published in 2024, microvesicles of 5 billion MSCs were applied in conjunction with sural autograft for repair in a case example of left radial nerve damage. At the 10th week, findings were obtained that the nerve was reinnervated. It was shown electroneurophysiologically that the patient still maintained regeneration at the

end of the 6th month[136]. Bioink forms can be produced by combining gel systems with various biomaterials that trigger neuronal differentiation, such as graphene. The cells or exosomal structures to be carried in these injectable systems are promising, especially in surgical applications such as nerve ending bonding[128].

In a study conducted by Giovannelli et al. in 2023, after creating a mouse and neuron traumatic brain injury model, it was observed that after mesenchymal exosomal treatment, mitophagy was activated by mediating the putative kinase protein 1/Parkinson protein 2 E3 ubiquitin-protein ligase pathway. Thus, it was observed that it reduced neuronal cell death and suppressed apoptosis, pyroptosis, and ferroptosis[137]. The use of exosomes in drug delivery systems is also a topic of interest, especially in neurology. When exosomes were loaded with dopamine and compared with control groups on cells in the *in vitro* 2D and 3D Parkinson's disease model, it was observed that EXO/DOP formulations improved proliferation, increased survival and neuroprotective activity[126]. Its activity was tested, and its neuroprotective activity was interpreted by artificial intelligence. As can be understood from here, the production of exosomes of approximately 100 nm size obtained from WJ-MSCs has become the focus of attention with their neuroprotective activities and ability to cross the blood-brain barrier[130,138].

Urology field

Erectile dysfunction (ED) affects sexual function and the capacity to sustain an erection[139]. To effectively treat ED, it is crucial to use a therapeutic strategy that fosters blood vessel growth, safeguards nerves, and supports tissue regeneration[140]. Various medications have been employed to manage this condition, with phosphodiesterase type-5 inhibitors being the most frequently prescribed by healthcare professionals[139,140]. Stem cell technology shows promise in treating ED. Specifically, MSC secretome therapy leverages the paracrine effects of stem cells, eliminating the risk of tumor formation and offering a minimal immune response. This method presents a significant potential solution for ED treatment, overcoming limitations of traditional therapies[141]. In 2010, Bahk et al. conducted the first human clinical trial using allogeneic umbilical cord MSCs to treat ED in patients with diabetes mellitus[142]. The study included seven men, aged 57-83, who received an intracavernous injection of 1.5×10^7 cells. Within 1 month, most participants experienced a return of spontaneous morning erections, and these improvements were sustained throughout the 6-month follow-up period[142]. Yiou et al. demonstrated that a single intracavernous injection of autologous BMMSCs was both safe and effective in treating vasculogenic ED[143] In a randomized controlled trial (RCTs), Mirzaei et al. demonstrated that an intracavernous injection of autologous MSCs ($5\text{-}6 \times 10^7$) significantly improved sexual function in the majority of diabetic patients[144]. Protogerou et al. developed a technique that integrates adipose tissue-derived MSCs (AT-MSCs) with components of the stromal vascular fraction[145]. They administered cultured AT-MSCs suspended in platelet lysate plasma and observed significant improvements in erectile function at 1, 3, and 6 months post-treatment, with no reported adverse effects.

Recent advances in understanding the pathology and molecular mechanisms of urinary incontinence have opened the door to exploring stem cell therapy as a potential solution for restoring continence[146]. Over 30 prospective single-center and multicenter studies, including follow-up assessments, have been conducted to evaluate the feasibility, safety, and efficacy of cell therapy in treating female stress urinary incontinence (SUI)[147]. Mahboubeh et al. reported that periurethral injections of autologous adult mucosa-derived stem cells were not inferior to the less invasive mini-sling procedure. The stem cell group experienced shorter intervention times, reduced hospital stays, and fewer complications. This pilot randomized trial demonstrated that stem cell therapy and mini-sling surgery produced comparable results in medium-term follow-up[148].

Approximately 1% of all males and 10-15% of infertile men are affected by azoospermia, a condition characterized by the complete absence of sperm in the ejaculate, as determined through the analysis of centrifuged semen samples[149]. In a study, a single intratesticular injection of pure MSCs was administered to treat patients[86]. Among the 87 azoospermia patients treated, 18 (or 20.7%) exhibited the presence of sperm in their semen at various intervals post-treatment. In a phase I clinical trial, an innovative treatment approach using autologous BMMSCs was developed for NOA (87). The study included 80 patients, 40 receiving BM-MSC therapy and 40 undergoing hormone therapy. BM-MSCs were transplanted into the testicular network using microTESE. After 6 months, improvements in hormone levels and sperm concentration were observed. Notably, 9 patients (22.5%) with secondary infertility and azoospermia showed successful outcomes with this treatment. The protocol and infusion method for stem cell application in patients with azoospermia are

illustrated in figure. 4. Stem cell therapies, particularly those involving mesenchymal MSCs, offer promising advancements in treating conditions such as ED, SUI, and NOA in urology area. These approaches demonstrate significant potential by improving outcomes and addressing limitations of traditional treatments, with minimal side effects.

Ophthalmology field

The studies on stem cells and exosome application in areas such as age-related macular degeneration (AMD), corneal damage and diseases, diabetic retinopathies, glaucoma, and various autoimmune eye diseases have become quite current and interesting. There are still controversial points regarding the dosage applications, toxicity status, and standardization of target-oriented transport systems of exosomes produced for these areas[150].

It has been stated that human exosomes of MSCs alleviate retinal ischemia by preventing retinal thinning against oxygen-induced retinopathy induced in mouse models[151]. MicroRNA-126-transferred MSC exosomes were shown to effectively suppress hyperglycemia-induced retinal inflammation both at the gene expression level and by retinal immunohistochemical section examinations[152].

There are studies suggesting that exosomes released from retinal astroglial cells that form the blood-retinal barrier can reduce retinal vascular leakage and that they contain anti-angiogenic components, which are a new treatment model for age-related macular degeneration. Recently, periocular injection models have been developed by taking more advantage of the anti-inflammatory properties of exosomes[153].

In a study, the neuroprotective and neuritogenic effects of BMMSCs-derived exosomes were investigated in a rat optic nerve crush model after weekly intravitreal application. It was observed that exosomes provided regeneration of retinal ganglion cells and preservation of axonal integrity[154].

Corneal damage, one of the causes of visual impairment, is identified by corneal opacity. In a study conducted in 2022, exosomes obtained from iPSCs were loaded onto time-release heat-sensitive chitosan hydrogels and both miRNA release profiles and regional cell regeneration were examined[155].

In another study, it was reported that in a mouse model of retinal ischemia-reperfusion injury, exosome-loaded degradable polymeric biomaterial poly(lactic-co-glycolic acid) microcapsules caused retinal thickness to return to a level close to the healthy retina control group with prolonged release[156].

In addition, topical applications can also be used. Spray products with nano-sized exosomes, cellular or biomaterials, increased area of effect and long-term release can be produced. The important advantages of these products are that they prevent infection formation and reduce the need for intravitreal or extra-ocular injections. It is thought that the use of these new generation regenerative products with high added value will increase, especially in common diseases such as glaucoma.

Previous studies have explored various routes for stem cell administration, including subretinal, suprachoroidal, and intravitreal approaches[157-162]. For instance, Both Oner et al. and Limoli et al. utilized adipose tissue-derived stem cells (ADSCs) delivered through the suprachoroidal route[161,162]. Öner et al. reported significant improvements in best-corrected visual acuity (BCVA) at the 6-month follow-up[161]. Importantly, no adverse effects, such as proliferation, rejection, or severe ocular or systemic complications, have been documented in these ocular applications[161-163]. Patients with dry AMD demonstrated improvements in BCVA at 6 and 12 months post-stem cell therapy compared to baseline, indicating its potential efficacy[163]. Subgroup analyses revealed variable BCVA outcomes based on stem cell types, such as human embryonic stem cell-derived retinal pigment epithelium, ADSCs, and CD34+ bone marrow-derived stem cells[163]. A meta-analysis of RCTs further supports the potential of SCT to enhance BCVA in patients with dry AMD[163].

Cardiovascular diseases field

Ischemic heart disease (IHD), also known as coronary heart disease or coronary artery disease, is one of the major causes of heart failure (HF), especially in developed countries[164-166]. Various strategies are being pursued to protect the injured myocardium and to prevent adverse cardiac remodeling[167-169]. Surgical revascularization has been shown in several studies to be an effective and preferred treatment strategy for patients with IHD[170]. Song et al. demonstrated that autologous bone marrow stem cell transplantation, in combination with coronary artery bypass grafting (CABG), improves left ventricular function compared to CABG alone[170,171]. In these patients, stem cells can be administered either intramyocardially (IM) or intracoronarily (IC)[172,173]. Qi et al. performed bypass surgery using the saphenous vein and injected approximately

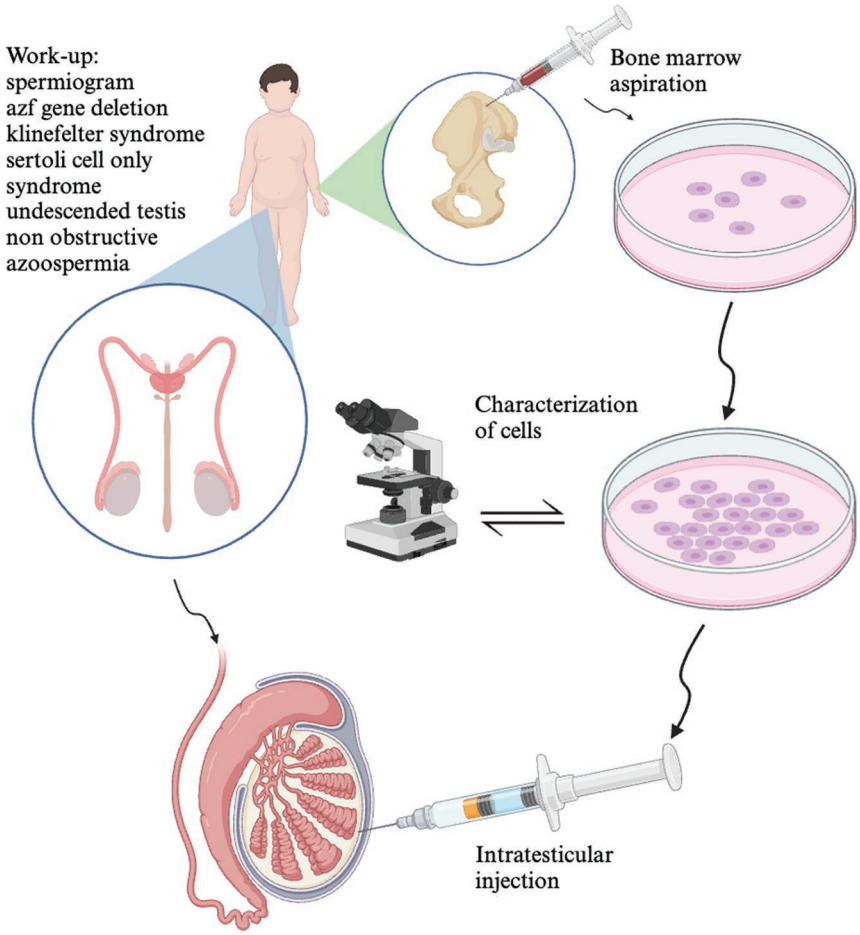

Figure 4. *Stem cell application model for azoospermia patients (injection into testicular parenchyma).*

Table 1. Differences in composition and size between ADEs and PDENs

Paramaters	Animal-derived exosomes	Plant-derived exosome-like nanoparticles	References
Size (nm)	30-150	50-500	9,20
Proteins	Membrane transporters (Fusion proteins, chaperones, cytoskeletal proteins) MVB synthesis proteins (tetras panin proteins, cell adhesion molecules, MHC molecules)	Cytosolic proteins (actin, proteolysis enzymes) membrane transporters (aquaporin, chloride channels)	21-23
Lipids	Cholesterol sphingomyelin glycosphingolipid phosphatidylserine	Phosphatidic acid phosphatidylethanolamin phosphatidylcholine digalactosyl monoacylglycerol digalactosyl diacylglycerol monogalactosyl diacylglycerol	24,25
Nucleic acids	DNA mRNA miRNA mtDNA lncRNA	DNA mRNA miRNA non-coding RNA	26-28

ADEs: Animal-derived exosomes; PDENs: plant-derived exosome-like nanoparticles; MVB: multivesicular bodie; MHC: major histocompatibility complex.

10^7 autologous BMMNCs through IC through a 1-min infusion during the procedure[172]. This method resulted in a greater improvement in the left ventricular dyssynchrony compared to CABG alone. On the other hand, Ulus et al. administered 70 × 10^7 autologous BMMNCs to one group and 25 × 10^6 allogenic

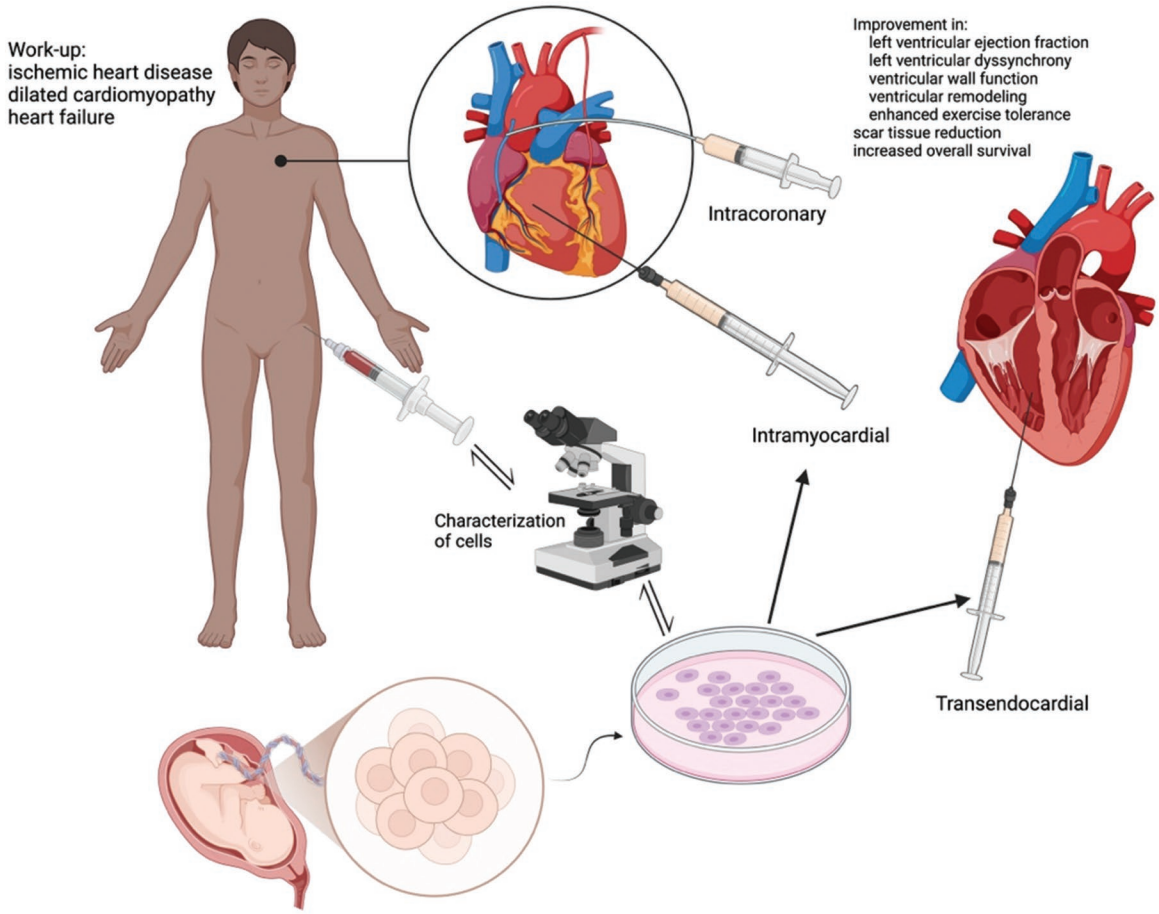

Figure 5. *Stem cell application model for cardiovascular disease (injection route: intracoronary, intramyocardial, transendocardial, and intravenous).*

UC-MSCs to another, both through IM injection into ischemic areas[173]. They observed that IM administration of UC-MSCs combined with CABG resulted in greater scar tissue reduction and improved ventricular wall function compared to autologous BMMNCs. However, the administration route and dose of stem cells remain controversial, requiring further research to its their efficacy and safety[171].

Despite advances in pharmacological treatments and implantable cardiac devices, clinical outcomes for HF patients remain suboptimal, highlighting the need for innovative therapies[174-176]. Various types of stem cells have been increasingly utilized in recent years to enhance clinical outcomes in HF patients[177]. A meta-analysis of 36 RCTs concluded that both BMMNCs and MSCs effectively improve left ventricular ejection fraction (LVEF) in patients with heart failure, with no significant difference observed between the two cell types[177]. Stem cell injection routes included intracoronary, intramyocardial, transendocardial, and intravenous. All routes significantly improved LVEF, while transendocardial injection showed superior outcomes for reducing major adverse cardiac events[177].

Although, some RCTs in patients with dilated cardiomyopathy (DCM) indicated that stem cell therapy did not significantly improve left ventricular function in cases of non-ischemic DCM[178-182], on the other hand, a systematic review and meta-analysis of 11 RCTs involving 637 patients indicated that stem cell therapy may have a beneficial effect on the prognosis of patients with non-ischemic DCM[183]. Another RCT involving 55 patients demonstrated that IC stem cell administration improved ventricular remodeling, enhanced exercise tolerance, and increased overall survival rates in patients with DCM[184]. The optimal type and quantity of stem cells, along with the most effective route of administration, remain contentious issues in the field of regenerative medicine for cardiovascular diseases. It is

important to note that these challenges highlight the need for further research, more detailed analyses, and large multicenter studies to establish reliable and effective therapeutic strategies. The methods of stem cell administration in cardiovascular diseases are illustrated in figure 5.

This review highlights the promising potential of stem cells and exosomes in regenerative medicine and surgical applications, yet certain limitations must be addressed. The heterogeneity in study designs, including variations in cell types, dosages, and administration routes, complicates the establishment of standardized protocols and hinders the comparability of findings. While initial results are encouraging, robust long-term safety and efficacy data remain scarce, limiting clinical translation. Furthermore, the lack of consensus on the optimal stem cell type, quantity, and administration route adds complexity to defining effective therapeutic strategies. Ethical concerns, such as equitable access and cost considerations, are also underexplored. To address these challenges, future research must focus on multicentric RCTs with standardized methodologies to ensure safe and effective integration into clinical practice.

Conclusion

Stem cell-based therapies have demonstrated significant success and acceptable safety profiles with in various used surgery to date. The advances in MSCs therapies, particularly those involving genetic modifications such as gene transfer or transduction, have shown promise in enhancing therapeutic outcomes. For example, strategies leading to overexpression of anti-inflammatory cytokines or inhibition of pro-inflammatory cytokines as well as modifications such as addition of pro-inflammatory cytokines to the culture medium have been effective in optimizing the therapeutic potential of MSCs. In addition, techniques to improve the homing ability of stem cells or exosomes, including the addition of surface markers, have further amplified their efficacy. Despite these promising developments, there are still challenges that may impede the widespread clinical translation of these therapies. Potential therapeutic risks, such as tumorigenesis and the high costs associated with these advanced treatments, remain significant concerns. Future clinical trials should therefore prioritize addressing these risks by focusing on strategies to minimize heterogeneity, determining the optimal routes of infusion, and establishing standardized protocols for dosage and infusion frequency. In addition, there is a need for the production of stem cells and exosomes under GMP conditions, their effective and safe application in the clinic, optimization of production and application protocols, and support with RCTs. The studies examined in this review include studies emphasizing the safety and efficacy of these treatments in comprehensive and diverse patient populations. Such studies are very important for determining therapeutic strategies and ensuring the safe integration of innovative exosome and stem cell-based therapies into standard clinical practices.

Funding

The authors declare that they have not received funding.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor requires ethical approval. The SAGER guidelines do not apply.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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