CELLULAR TRANSPLANTS (G ORLANDO, SECTION EDITOR)

Extracellular Vesicles, Apoptotic Bodies and Mitochondria: Stem Cell Bioproducts for Organ Regeneration

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Abstract

Purpose of Review In the current work, we will present the characterization of the main different stem cell-derived vesicular bio-products with potential application in organ regeneration.

Recent Findings The therapeutic effects of stem cell therapy in organ repair, specifically those utilizing mesenchymal stromal cells, are largely dependent on the cells' release of different bio-products. Among these bio-products, extracellular vesicles (EVs) appear to play a major role due to their ability to carry and deliver bioactive material for modulation of cellular pathways in recipient cells. Concurrently, mitochondria transfer emerged as a new mechanism of cell communication, in which the bioenergetics of a damaged cell are restored. Finally, apoptotic bodies released by dying apoptotic stem cells contribute to stimulation of the tissue's stem cells and modulation of the immune response.

Summary Exploitation of isolated extracellular vesicles, mitochondria and apoptotic bodies in preclinical models of organ damage shows promising results. Here, we describe the results of the pre-clinical applications of stem cell vesicular products, as well as the first clinical trials approaching artificial administration of extracellular vesicles and mitochondria in human subjects and their possible benefits and limitations.

Keywords MSC · Regenerative medicine · MicroRNA · Exosomes · Microvesicles · Mitochondrial transfer · Apoptosis

Introduction

Organ failure is the most frequent cause of morbidity and mortality recorded in Europe and in the United States in recent decades. Organ dysfunction can be attributed to fibrosis, a pathological feature of many chronic inflammatory diseases, as its extensive remodelling of tissues leads to functional insufficiency [1]. The burden associated with fibrosis is disconcerting, representing in the United States almost half, and in

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the industrialized world about 30%, of all deaths attributed to fibrotic heart, lung, kidney and liver diseases [1, 2]. In addition, episodes of acute tissue injury, especially if severe and repeated, are closely associated to development of chronic organ disease [3].

It has therefore become of increasing interest in Regenerative Medicine to limit the progression of fibrosis, promote restoration of organ function in chronic settings and support organ repair after acute injury to regain tissue integrity. In this context, increasing studies underline the role of stem cell bio-products, including secreted soluble factors and extracellular vesicles (EVs), as powerful instruments in organ regeneration. EVs, in particular, have been proposed as a new form of intracellular messaging through their ability to reach distant organs and deliver the active cargo necessary for reprogramming of the target cells. In addition, EVs released by apoptotic cells, including apoptotic bodies (ApoBDs), are recently emerging as part of the therapeutic and immune-modulating mechanisms of injected stem cells within injured tissues [4] (Fig. 1).

Finally, stem cell therapy involves the transfer of mitochondria, the organelles responsible for cellular energy production, from stem cells to damaged cells. Mesenchymal stromal cells

Fig. 1 Presentation of apoptotic and healthy cell secretome
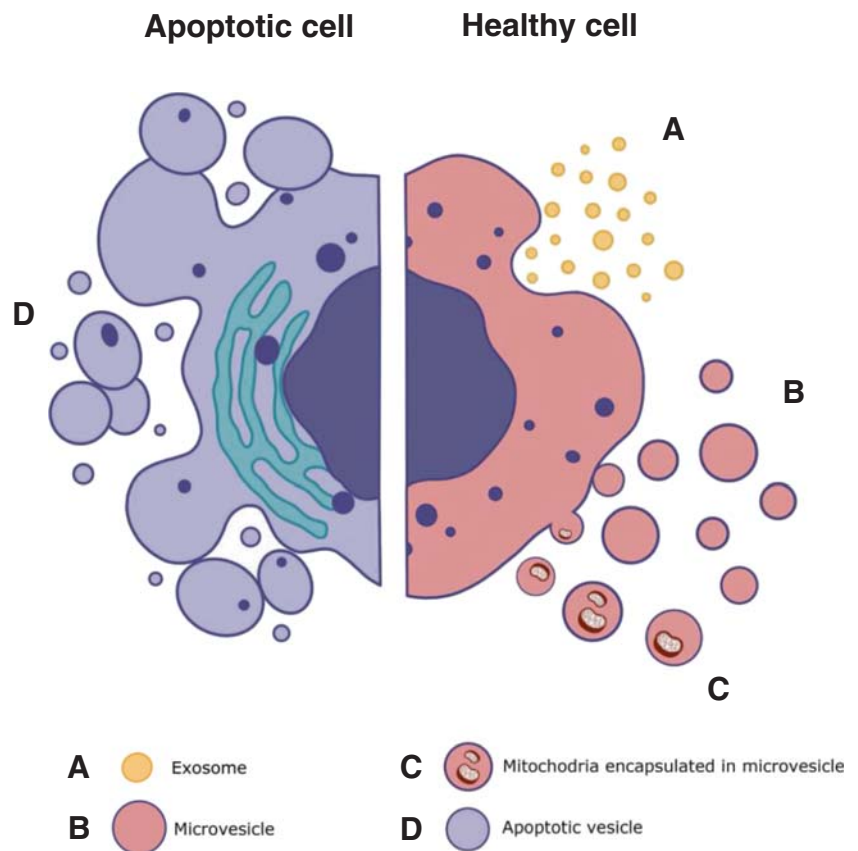
(MSCs) are shown to transfer mitochondria to the recipient cells in different ways: encapsulated within EVs [5]; *via* cell-to-cell direct communication through tunnelling nanotubes; or through direct release of "naked" mitochondria into the extracellular microenvironment [6]. The organelle incorporates into the endogenous mitochondrial network of the damaged recipient cell that needs to be rescued, restoring its bioenergetic profile and health [7].

In this review, we will present the recent knowledge on mechanisms of action involved in the therapeutic effects of healthy and apoptotic EVs, as well as of mitochondria transfer, and the exploitation of these bio-products in preclinical models of organ damage. Finally, we will describe the first clinical trials approaching their use on human subjects and the possible benefits and limitations.

Extracellular Vesicles

Since the 'discovery' of EVs in blood plasma in 1946 by Erwin Chargaff and Rudolph West [8], interest in those cells-to-cell communicators has risen in almost all fields of biology and chemistry. EVs have been proven to naturally occur in prokaryotes, eukaryotes, plants and cells. EVs are membrane-bound, spherical particles enclosed in a lipid bilayer. In biological samples, EVs originate

from their parental cell, taking up their internal and external composition. Guidelines from the International Society for Extracellular Vesicles (ISEV) classify three main categories of EVs; exosomes also named small EVs (~30–250 nm), large EVs or microvesicles (~100–1000 nm) and ApoBDs (>1 μm) [9••]. Small EVs and microvesicles are released from metabolically active cells, whereas ApoBDs are exclusively produced during cell apoptosis [10] (Fig. 1).

The content of an EV is dependent on its origin, size and the route of biogenesis. EV surface markers and cargo are specific to the three types of vesicles (Table 1) and are most commonly associated with the route of vesicle formation. The process of exosome formation begins with inward budding of early endosomes and formation of intraluminal vesicles; this involves the ESCORT complex, ALIX and tumour susceptibility gene 101 (TSG101), all of which are responsible for cargo sorting. Intraluminal vesicles mature into multi-vesicular bodies, followed by fusion with the cell membrane and release of vesicles into the extracellular environment. Alternatively, multi-vesicular bodies are degraded by lysosomes and their components recycled. Exosomes are distinguished by the presence of all three tetraspanin markers responsible for induction of membrane curvature (CD9, CD63 and CD81). Proteins that can be detected and are involved in exosome biogenesis include Rab, GTPases, annexin, flotillin,

Table 1 Composition, size and biogenesis route of EVs

Vesicle type	Origin	Size	Markers	Components
Exosomes	Endolysosomal pathway; fusion of MVB with cell membrane	30-250 nm	CD9, CD63, CD81 ESCORT components, TSG101, flotillin, Annexin	mRNA, miRNA and other non-coding RNAs; membrane and cytoplasmic proteins, lipids, receptors
Microvesicles	Cell membrane; bud off directly from cell surface	100-1000 nm	CD40	mRNA, miRNA and other non-coding RNAs; membrane and cytoplasmic proteins, lipids, receptors
Apoptotic bodies	Cell Membrane; membrane blebbing during apoptosis	>1μm	Phosphatidylserine, Calreticulin, Calnexin	Nuclear fragments and cell organelles

ALIX, TSG101, VPS4, heat shock protein (HSP70) and the ESCORT complex [11].

Microvesicles are formed by direct budding from the cell plasma membrane with involvement of cytoskeleton components and fusion machinery, though the process is not yet fully understood. Due to their biogenesis pathway, microvesicles are primarily composed of a plasma membrane and of cytosolic-associated proteins. Other commonly found components include heat shock proteins, integrins, post-translationally modified proteins and RNA species. Several structural components are shared between exosomes and microvesicles due to their similar release pathways and origin [11].

EVs have been designated as novel cell-to-cell communicators due to their effect on cells on a paracrine and endocrine level, specifically through the direct stimulation of cell surface receptors and transfer of bioactive molecules. Indeed, EV surface receptors may act as signalling complexes and directly stimulate target cells or, alternatively, transfer functionally active receptors from one cell to another. For instance, bystander B cells can acquire antigen receptors from activated B cells becoming specific activated antigen presenting cells for CD4 T cells [12].

In addition, the presence of a complex cargo (miRNA, RNA, proteins, lipids, cytokines and mitochondria) within EVs results in a multilevel modulation of cell functions in the recipient cells [13, 14]. Small RNA species, including miRNA, are present within EVs and, interestingly, recent studies found that the overall pattern of miRNA content of small and large EVs appears similar but distinctly different from that of the originating cells [15••], implying specific mechanisms of miRNA packaging into EVs. Moreover, extensive proteomic studies on EVs originating from cell cultures, tissue cultures and isolated bio-fluids have shown a significant EV protein content. Online databases created through the collaboration of EV research groups provide us with catalogued EV components, such as Exocarta, (www.exocarta.org), EVpedia (www.evpedia.info) and Vesiclepedia (www.microvesicles.org). EVs contain many common proteins involved in vesicle trafficking and serving as part of the cytoskeleton and the plasma membrane. Furthermore, specific protein content reflects the EV mechanism of generation and origin, as well as the cellular state of the EV originating cell. Finally, in addition to the structural functions of lipids in EV membranes, bioactive lipids such as eicosanoids, fatty acids and cholesterol are transferred by EVs to recipient cells. For instance, sphingomyelin has been shown to regulate angiogenesis in vitro and in vivo in tumour derived EVs [16].

Apoptotic EVs

Apoptosis is commonly known as programmed cell death. An apoptotic cell undergoes several morphological changes: membrane blebbing, membrane protrusion formation and generation of ApoBDs [17–19]. The membrane of ApoBDs reflects the main changes occurring in the cell surface of the apoptotic cell. In particular, apoptotic cells express markers promoting their removal by surrounding cells or macrophages before the cell membrane ruptures [20]. For instance, Calreticulin, an "eat me" ligand is physiologically silenced by the CD47 "don't eat me" ligand; and only expressed by cells and ApoBDs when CD47 is downregulated [21]. The size of ApoBDs ranges from 1 μm to 5 μm [22, 23]. This characteristic is similar to oncosomes (EVs secreted by cancer cells), but the biogenesis of these vesicles differs [24]. The number of ApoBDs produced per cell was quantified as 12.87 ± 3.23 per hour [25••]. In comparison, the average number of released EVs by mesenchymal stem cells was found be in the range of 2900 per cell, overnight [26].

During apoptosis, apoptotic microvesicles 0.1–1 μm in diameter and small exosome-like EVs are released [27, 28]. However, these vesicles are less characterized than the ApoBDs.

ApoBDs are characterized by the presence of externalized phosphatidylserine and by a permeable membrane. As mentioned above, they express phagocytosis-promoting signals, such as calreticulin [21] and calnexin [29]. In addition, ApoBDs express chemokines and adhesion molecules, such as CX3CL1/fractalkine and ICAM3, and MHC class II

molecules, allowing for direct antigen presentation to CD4^{+} T cells and activation of immunological memory [30]. The cargo of ApoBDs consists of cellular components enclosed during protrusion. Due to this fact, the content of ApoEVs can be very diverse. Indeed, ApoBDs can contain microRNAs, RNA and DNA. Diversity of ApoBDs content affects their physiological properties. ApoBDs can be subdivided into two groups: DNA-carrying ApoBDs and cytoplasm-carrying ApoBDs. 5′ phosphorylated blunt-ended DNA can be used as a distinctive marker of DNA-carrying ApoBDs because it is exclusively found in ApoBDs, which undergo apoptosis and contain the DNA fragments [31].

Mitochondria

While mitochondria are widely considered the powerhouse of the cell, as they are responsible for ATP production through oxidative phosphorylation, these organelles are also involved in several other pathways. They serve a role in pluripotent stem cell maintenance [32], apoptosis and cell death regulation and proliferation capacity through complex interactions between p53 and reactive oxygen species (ROS) production [32]. Energy deprivation and mitochondria dysfunction has been strictly associated with end stage organ disease [33]. Therefore, while metabolic patterns and mitochondria contents can strictly vary among the organs, it appears evident that mitochondrial alterations are closely correlated with most of the clinical conditions that lead to organ failure [34, 35].

As mitochondria do not possess an efficient DNA-repair system [36], these organelles are typically recycled through mitophagy, a form of autophagy [37]. Moreover, the transfer of respiration-competent mitochondria from cell to cell emerged in the past few years [6] as a mechanism of damage repair or cell reprogramming [38]. The physiological mitochondria transfer is a biological phenomenon in which the organelle from a healthy donor cell is relocated into a stressed recipient cell, resulting in repair and survival of the damaged cell. During this process, the mitochondria's small size and plasticity allow it to be transported from donor to recipient cells through transporting mechanisms, such as tunneling nanotubes and microvesicles. The organelle is eventually incorporated into the endogenous mitochondrial network of the recipient cell that needs to be rescued, restoring the cell to its bio-energetic profile and health [39].

The incorporation of respiration-competent mitochondria within released microvesicles has been extensively studied in MSCs, where, once internalized, mitochondria-containing microvesicles can rescue cells from injury or act as reprogramming factors [5, 40••]. In 2012, Islam et al. demonstrated that mitochondria can be transferred in vivo through a mouse model of acute lung injury [38]. Firstly, mitochondria-labelled MSCs were administered by injection into the damaged lungs. These MSCs homed in the damaged tissue and produced microvesicles containing the labelled mitochondria 4 h post-injection. The microvesicles were then directly transferred into the damage lung cells, resulting in the restoration of their ATP concentrations and secretory responses [38].

PMT has also been observed through the establishment of tunnelling nanotubes (TNTs) [6]. TNTs are filamentous connections formed by protrusions of a cell's membrane and are used to share organelles and contents of the cell's cytoplasm with other cells [41]. The utilization of TNTs in PMT has been observed in transfers between MSCs and macrophages, which served to enhance macrocytosis and activate antimicrobial response [42]. Moreover, Sinclair et al. demonstrated that the transfer of mitochondria via TNTs is essential due to the regenerative capacity exerted by MSCs [41]. However, the role and mechanism of PMT through the establishment of TNTs is poorly understood. The last and least known mechanism of PMT is the direct release of mitochondria in the extracellular microenvironment, usually in response to cell stress. "Naked" mitochondria can be encapsulated in a vacuole and then extruded by apoptotic cells, specifically hepatocytes [43]. Similarly, platelets can release respiration-competent mitochondria as a mediator of innate immune response [44].

Therapeutic Effects of EVs, ApoBDs and Mitochondria for Organ Regeneration

EV Reprogramming of Injured Tissue

Following the emerging interest of stem cell therapy, an increasing number of research and pharmaceutical groups are focusing on stem cell derived EVs, specifically MSC-derived EVs, as a new form of therapeutic agents for organ regeneration and protection [45–48]. Due to the ability of MSC-derived EVs to transfer therapeutic molecules, such as mRNAs, miRNAs and protein, and several of their regenerating effects to MSCs, this source of EVs is one of the most studied in Regenerative Medicine. Moreover, MSCs produce a higher number of EVs in comparison with other stem cells [49]. MSC-derived EVs were proven to modulate the immune system and stimulate regeneration in a multitude of preclinical models, including graft-versus-host-disease, lung, liver, kidney and cardiovascular injury [45]. There are a significant number of studies describing the pro-regenerative effects of stem cell-EVs for organ regeneration, but it is not within the scope of this review to detail all the different preclinical models of application.

As detailed above, the therapeutic effect of stem cell-EVs on organ repair is related to transfer of pro-regenerative proteins or microRNAs. For instance, although numerous factors have demonstrated the therapeutic effects of different EV models and origins, not a single agent emerged as pivotal or

indispensable. Therefore, it can be hypothesized that not a single factor, but rather a synergic and multi-target action of EV components is responsible for the therapeutic EV results. Indeed, the common and characterizing action of therapeutic MSC-EVs can be described as a reprogramming activity on tissue expression profiles. For instance, in models of kidney and liver injury [26], the expression profile of the EV-treated diseased organ, as assessed by RNA sequencing, correlated with that of the normal tissue. Moreover, in models of chronic tissue injury, an upregulation of anti-fibrotic genes and downregulation of pro-fibrotic genes was common to the different diseases as well as stem cell sources [50, 51]. Through modulation of their phenotype and subsequent secretomes, therapeutic utilization of EVs may benefit from an in vitro stimulation or manipulation of the generating cell source. For instance, ischemic or hormonal stimulation may ameliorate EV activities [52–54]. In addition, EV administration for chronic diseases might require multiple administrations. The possible development of immune reactions in this setting has not yet been studied in depth.

Regenerative Effect of Apoptotic Body Phagocytosis

Due to the rapid clearance of damaged cells by immune cells, namely phagocytes, ApoBDs play a major role in immune regulation [30, 55]. ApoBDs are emerging as a pivotal tool in cell-to-cell communication between damaged and healthy cells, therefore modulating mechanisms of organ repair. Indeed, ApoBDs may stimulate proliferation of resident stem/progenitor cells, improving tissue regeneration and replacing damaged cells [25••, 56]. For instance, phagocytosis of the ApoBDs by hepatic stellate cells can promote their differentiation and increase their cell survival [57]. Moreover, ApoBDs' engulfment may support MSC homeostasis. In particular, systemic infusion of exogenous ApoBDs was able to rescue apoptotic MSCs by transferring RNF146 and miR-328-3p and activating the Wnt/β-catenin signalling [58•]. In parallel, in zebrafish, dying epithelial stromal cells of the epidermis were observed to generate Wnt8a enriched ApoBDs, supporting the hypothesis of ApoBDs being biologically active vehicles in cell-to-cell communication [25••]. Neighbouring p63-positive stromal cells engulfed the ApoBDs, which caspase-dependently activated Wnt signalling and stimulated cell proliferation and tissue homeostasis over 24 h. In this model, inhibition of apoptosis significantly reduced the number of proliferating stromal cells. On the contrary, overexpression of the Wnt pathway in combination with apoptosis induced a significant increase of stromal cell proliferation [25••].

ApoBDs can deliver microRNAs, DNA and other genetic material to target cells, resulting in a multitude of different effects. For example, miRNA-126, present in endothelial ApoBDs, promoted chemokine CXCL12 expression in healthy endothelium, and repeated administration of those

ApoBDs in mice with atherosclerosis induced an athero-protective effect [46].

Although the use of ApoBDs generated in culture as therapeutic has not yet been tested, their role appears of increasing interest in the field of Regenerative Medicine.

Artificial Mitochondria Transfer

In recent years, artificial mitochondrial transfer (AMT) emerged as a new possible therapeutic option for tissue repair. AMT has been intensively investigated in cardiac disease models. In a rabbit model of cardiac IRI, the injection of viable respiration-competent mitochondria, isolated from donor rabbit cardiac or muscular tissues, was able to significantly reduce the infarct size area, kinase MB, cardiac troponin-I and apoptosis in the regional ischemic zone [47, 48]. AMT has also been recently tested in a mouse model of heterotopic heart transplantation: mitochondria isolated from gastrocnemius muscle were autologously administrated in the heart coronary ostium before and after the transplant. Within 24 h after transplant, necrosis and neutrophil infiltration were significantly decreased compared with the vehicle-treated group. Moreover, the mitochondrial treatment significantly enhanced the beating score after transplant [59]. Interestingly, these papers demonstrated both in vitro and in vivo that fully differentiated cells can be used as a source for the mitochondrial injection.

Moreover, transfer of mitochondria, mainly derived from MSCs, has been proven effective in other pathologic models involving liver, brain and kidney. In a rat model of liver IRI, the intra-splenic administration of MSC mitochondria mitigated the necrosis of hepatocytes as well as reduced the expression of mitochondrial-induced apoptosis markers [60]. In the kidney, the effectiveness of AMT was demonstrated by rescue of damaged renal proximal tubular cells. In vitro, the administration of MSC-derived mitochondria reduced ROS production and increased the expression of the tubular marker megalin and mitochondrial superoxide dismutase 2, whereas in vivo, both the tubular basement membrane and brush border were protected [61]. Moreover, in normal mice, the administration of mitochondria improved endurance during forced swimming test. Finally, AMT has also been used in vivo in a murine model of Parkinson's disease and in vitro for the regeneration of damaged hippocampal cells [62, 63].

Although of great novelty, these therapeutic approaches have already started to be applied in the human setting.

Clinical Trials Involving EVs and Mitochondria Transfer

All the clinical trials concerning MSC-EVs and ATM can be found at www.clinicaltrails.gov. Although the majority of listed trials focus on the diagnostic properties of EVs, there

are five trials testing the therapeutic applications of MSCs-EVs and two proposing the use of ATM (Table 2).

A first trial is designed to test the anti-inflammatory properties of umbilical cord derived MSCs-EVs to prevent the destruction of pancreatic β-cell islets. The MSCs-EVs will be administered intravenously in two doses, the first dose of exosomes and, after seven days, the second dose of microvesicles (NCT02138331). Two other clinical trials using EVs will involve allogeneic MSCs-EVs. One of them will administer EVs enriched by miR-124 for the treatment of acute ischemic stroke (NCT03384433). The second clinical trial will attempt to treat lesions in patients affected by dystrophic epidermolysis bullosa (NCT04173650). The last clinical trial using EVs that is in the recruiting phase focuses on promoting the healing and recovery of refractory macular holes through direct injection of MSC exosomes to the site of the injury (NCT03437759). Finally, the only concluded trail to date used umbilical cord derived MSCs-EVs to inhibit the progression of chronic kidney disease in patients with grade III-IV CKD [64]. The study showed stabilization of the disease progression, as confirmed by stable levels of glomerular filtration rate, serum creatinine and blood urea in treated patients, and an increased level of anti-inflammatory factors (TGF-β1 and IL-10) in comparison with the matching placebo group.

The first clinical trial using administration of isolated mitochondria for the treatment of myocardial IRI has also been concluded with positive results [65]. Mitochondria were isolated from non-ischemic skeletal muscles and injected in the myocardium of paediatric patients with myocardial IRI. No adverse effects were detected after AMT, and four out of five patients demonstrated an enhancement in ventricular function [65].

Other clinical trials using AMT are focused on the improvement of infertility treatments (Table 2). Through autologous micro-injection of mitochondria prior to intra-cytoplasmic sperm injection, the patients' oocyte quality was enhanced. In the first trial, concluded in 2017, mitochondria were isolated from autologous ovarian stem cells and directly injected into the oocytes themselves. To date, no results have been published. Embryo quality has been quantified through the pregnancy rate after treatment and morphological evaluation of the treated embryos. In the second clinical trial, which is still ongoing, mitochondria will be isolated from autologous bone marrow-MSCs and administrated immediately before intra-cytoplasmic sperm injection in the oocytes. Live birth

Table 2 Current clinical trials concerning the use of EVs or AMT as therapeutic agents

	Intervention	N. pts	Follow up	State	Location	Number/Ref.
Diabetes Mellitus type 1	Two doses of MSC-EVs	20	3 months	Unknown	Sahel Teaching Hospital Sahel, Cairo, Egypt	NCT02138331
Chronic kidney disease	Two doses of umbilical cord MSC-EVs (100 μg/kg/dose)	20	1 year	Concluded	Sahel Teaching Hospital Sahel, Cairo, Egypt	Nassar et al.
Molecular degeneration	20–50 mg of cord tissue MSC-EVs injected directly around macular hole	44	24 weeks	Recruiting	Tianjin Medical University Hospital Tianjin, China	NCT03437759
Cerebrovascular disorders acute ischemic stroke	Allogeneic MSC-EVs enriched by miR-124	5	12 months	Not recruiting yet	Shahid Beheshti University of Medical Sciences, Tehran, Iran	NCT03384433
Dystrophic Epidermolysis Bullosa	Allogeneic MSC-EVs applied directly to lesions, blisters for 60 days	30	2 months	Not recruiting yet	Aegle Therapeutics, Miami, Florida USA	NCT04173650
Repetition failure	Clinical Application of Autologous Mitochondria Transfer for Improving Oocyte Quality.	50	2-3 years	Not recruiting yet		NCT03639506
Infertility	Amelioration of oocyte quality using autologous mitochondria transfer	59	5 months	Concluded	Valencia, Spain	NCT02586298
IRI	Autologous mitochondrial transfer for dysfunction after ischemia-reperfusion injury	5	6 days	Concluded	Boston, MA, USA	Emani et al.

rate, pregnancy rate, number of oocytes retrieved and fertility rate are going to be evaluated.

Conclusion

Numerous discoveries within the Regenerative Medicine field have highlighted the bioactivity of stem cell bio-products and their role in cell-to-cell communication. In particular, EVs are the most advanced as potential therapeutic agents due to their ability to modulate the function of targeted cells. Together with stem cell-EVs, proven to be of therapeutic effect in a large variety of pre-clinical models, ApoBDs and AMT can be utilized for selected and specific applications.

The major issue with use of EVs in clinical practice is the standardization of EVs isolation, usage and storage. However, once those issues can be overcome, using EVs as therapeutic agents provides solutions to numerous complications caused by stem-cell therapy, including immune compatibility, maldifferentiation and tumourigenicity. EV therapy allows for dosage control, evaluation of safety and potency equivalent to pharmaceutical agents. In comparison to stem cell therapy, EVs are potentially an easier option as they can be directly obtained from the medium of cultured cells, massively produced and stored without the application of toxic cryopreservative agents and/or loss of EV potency [66]. The biological properties of EV allow for modification of the EV content to obtain desired cell-specific effects. Encapsulation of effector molecules (nucleic acids, lipids and proteins) by EVs allows delivery of its cargo without the risk of degradation. Genetically modified EVs may offer a more effective and natural solution than usage of FDA-approved lipid nanoparticles, which pose a low-dose toxicity upon cell entry [67].

The application of ApoBDs is still poorly explored, as several aspects still require further investigation. For instance, apoptotic vesicles are quite heterogeneous and may have different compositions and properties depending on their size. While EVs released by primary murine aortic endothelial apoptotic cells enhanced inflammation in mice transplanted with an MHC-incompatible graft, ApoBDs did not show this behaviour [28]. Therefore, as increasing experimental evidence suggests, the therapeutic effects of stem cells are due to their clearance by the immune system [4, 68]. For this reason, therapeutic utilization of these cell products appears of interest.

Finally, the use of MSCs as a source of mitochondria for AMT is a novel, therapeutic option that shows regenerative effects in the treatment of acute cell damage. However, more studies are required for better understanding of mitochondria internalization, their fate once inside the injured cells and how mitochondria can survive in the extracellular microenvironment or the blood flow during AMT.

Compliance with Ethical Standards

Conflict of Interest All authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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