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REVIEW ARTICLE

Extracellular Vesicles in Regenerative Medicine: Potentials and Challenges

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Abstract The ultimate goal of regenerative medicine is to regain or restore the damaged or lost function of tissues and organs. Several therapeutic strategies are currently being explored to achieve this goal. From the point of view of regenerative medicine, extracellular vesicles (EVs) are exceptionally attractive due to the fact that they can overcome the limitations faced by many cell therapies and can be engineered according to their purpose through various technical modifications. EVs are biological nanoscale vesicles naturally secreted by all forms of living organisms, including prokaryotes and eukaryotes, and act as vehicles of communication between cells and their surrounding environment. Over the past decade, EVs have emerged as a new therapeutic agent for various diseases and conditions owing to their multifaceted biological functions. This is reflected by the number of publications on this subject found in the Web of Science database, which currently exceeds 12,300, over 85% of which were published within the last decade, demonstrating the increasing global trends of this innovative field. The reviews collected in this special issue provide an overview of the different approaches being explored in the use of EVs for regenerative medicine.

Keywords Clinial trials · Commercialization · Extracellular vesicles · Regenerative medicine

EVs, the ubiquitous particles once considered as an elimination apparatus for cellular waste [1]. have added an additional layer to the conventional modes of intercellular communication, including direct cellular contact via adhesion molecules and soluble mediators (hormones, growth factors, cytokines and chemicals). It has become evident that these membrane-enclosed nanoscale particles (40–1000 nm), secreted by donor cells, exchange biological information between cells and participate in a diverse array of physiological and pathological processes [2]. The composition of these subcellular particles includes growth factor receptors, ligands, adhesion proteins, mRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), second messengers, metabolites, and lipids that reflect their cellular origin. The decorating proteins on the surface of EVs may serve as a type of postal code that delivers membrane-enclosed messages. In general, EVs are commonly divided into two major subgroups according to their size and biogenesis: microvesicles (MVs) and exosomes. MVs are 100–500 nm in diameter, are generated by budding off from the plasma membrane, and represent a subgroup of larger vesicles. Exosomes, which are much smaller vesicles with a diameter of approximately 40–150 nm, are formed by the reverse budding of endosomal multivesicular bodies and are secreted from cells upon the fusion of these bodies with the plasma membrane. It is difficult to obtain pure vesicle fractions of microvesicles and exosomes because of the size, density, and protein marker overlaps between microvesicles and

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exosomes. Due to the methodological difficulties associated with distinguishing these sub-groups, it has also been proposed to substitute the term "extracellular vesicles (EVs)" in accordance with ISEV 2018 guidelines [3].

Mesenchymal stem cells (MSCs) can be applied in regeneration, and have a long history of extensive basic research and beneficial results in clinical trials. Many preclinical studies have reported paracrine factors as key therapeutic agents for MSC-based cell therapies [4]. Among these paracrine factors, the therapeutic roles of EVs in regenerative medicine have been elucidated by studies utilizing animal disease models of kidney, musculoskeletal, cardiovascular, hepatic, neurological diseases and hair loss [5–10]. A recent study showed that MSCs-EVs ameliorated LPS-induced acute respiratory distress syndrome (ARDS) in a mouse model, indicating their utility in the control of the inflammatory response and fibrotic events following Covid-19 infection [11]. In addition to MSCs, embryonic stem cells, induced pluripotent stem cells, tissue-specific stem cells, progenitor cells derived from stem cells, and even terminally differentiated cells may also be successfully used in tissue regeneration as EV producers [12–14].

Accumulating evidence of preclinical therapeutic efficacy and their versatility in tissue repair and regeneration has brought attention to EVs as a potential regenerative substance. Although recent studies have shown that the regulation of apoptosis, cell proliferation, differentiation, migration, angiogenesis, oxidative stress, aging, and inflammation are mainly attributed to the action of EVs [15], the molecular biological mechanisms involved in EV-mediated tissue repair and regeneration have not been fully elucidated. Studies have suggested that three molecular entities in the EV composition play key roles in EV-mediated tissue repair and regeneration processes: miRNAs, mRNAs, and proteins. To date, several specialized signaling pathways related to regenerative processes, such as mitogen-activated protein kinase, Wnt/β-catenin, PI3K/Akt, Notch, TGF-β/Smad, STAT and Hedgehog signaling, CaMKII, and Efna3 signaling, have been identified upon EV stimulation [16–19]. EVs can deliver key proteins directly or control their upstream or downstream components by regulating gene expression with mRNAs or miRNAs [20], a subtype of small (19–24 nucleotides), non-coding RNA molecules that target mainly mRNA molecules to regulate gene expression at the post-transcriptional level. Many studies have evaluated the miRNA cargo of EVs and proposed their regulatory roles in cell proliferation, differentiation, and apoptosis during tissue regeneration. Several miRNAs act as potential contenders for tissues and organ-specific tissue regeneration. For example, miR-124 and miR-9/9* induce the direct conversion of fibroblasts into neuron-like cells by modulating chromatin remodeling complex [21], and miR-1 and miR-133a protects the myocardium against apoptosis, oxidative stress, and fibrosis and promotes cardiac regeneration [22]. Furthermore, the immunomodulatory role of EVs has been demonstrated by miR-146a in BM-MSC-derived MVs in allogenic kidney transplantation [23].

mRNAs are another prime messenger in EVs in tissue regeneration. In particular, the horizontal transfer of mRNAs from donor cells to recipient cells is evident in studies utilizing MSC-derived EVs [18]. The therapeutic action of MSC-EV-delivered mRNAs related to Gene Ontology terms of immune regulation and damage repair to recipient cells have already been documented in several studies [24]. For example, Choi et al. [25] found that MSC-EVs containing mRNA of vascular endothelial growth factor (VEGF-A), basic fibroblast growth factor (bFGF), and insulin-like growth factor 1 (IGF-1) induced the proliferation of peritubular capillary endothelial cells in acute renal ischemic mice. Additionally, the horizontal transfer of neuregulin 1 mRNA in adipose stem cell (ASC)-derived EVs diminished muscle damage and inflammation in a mouse model of hind limb ischemia [26]. However, it should be noted that the regenerative effect observed in this study is not solely manifested by the horizontal transfer of mRNA species by EVs. Proteins in EVs are known to modulate the intracellular and extracellular microenvironment of recipient cells. Proteome studies of MSC-EVs have identified proteins associated with tissue repair and regeneration via angiogenesis, coagulation, apoptosis, inflammation, and extracellular matrix remodeling [27, 28].

The accumulation of knowledge regarding EVs using disease models has provided potential opportunities for their clinical applications in a variety of diseases [29, 30]. Based on their compact size, collection efficiency, biocompatibility, and engineered production, EVs have many advantages as a therapeutic delivery tool for regenerative medicine. However, several regulatory hurdles and technical challenges must be addressed for the successful clinical translation of these remarkable biological particles. These include defining therapeutically active sub-populations of EVs among heterogeneous vesicles, the optimization of the purification step, scale-up production, dosage, route of administration, safety of EVs (toxicity, immune response, and pharmacodynamics), regulation of complications, and quality management [31, 32]. Although several clinical trials of EVs are in progress, majority are focused on biomarkers, pathological mechanisms, and cancer treatment, and only a few studies have focused on

Table 1 Lists of clinical trials using EVs for tissue repair and regeneration

Target tissue	Disease	Intervention	Trial purpose	Trial phase	Contry
Lung	Bronchpulmonary Dysplasia	BMMSC-EVs (UNEX-42)	NCT03857841	Phase I	USA
	Pneumonia by COVID-19	MSC-CM	NCT04798716	Phase I, Phase II	USA
	Acute Respiratory Distress Syndrome	ADMSC-EVs	NCT04602104	Phase I, Phase II	China
	Pneumonia by COVID-19	ADMSC-Evs	NCT04798716	Phase I	USA
	Pneumonia by COVID-19	COVID-19 Specific T cell-derived exosomes (CSTC-Exo)	NCT04389385	Phase 1	Turkey
Bone and cartilage	Osteoarthritis	ADMSC-EVs	NCT04314661	Phase I	Indonesia
	Low back pain	PRP-EXSOME	NCT04849429	Phase I	India
	Periodontitis	ADMSC-EVs	NCT04270006	Early Phase 1	Egypt
Muscle	Muscular dystrophy	Cardiosphere-derived Cells-Evs(CAP-1002)	NCT03406780	Phase 2	USA
Brain	Acute ischemic stroke	MSC-EVs	NCT03384433	Phase I, Phase II	Iran
	Alzheimer Disease	MSC-EVs	NCT04388982	Phase I and II	China
	Brain injury, Cognitive disorder	M2 macrophage-derived bioactive factors		Phase I and II	
	Craniofacial neuralgia	Exosome	NCT04202783	N.A	USA
Cardiovascular	Heart attack	PEP (EV-based product)	NCT04327635	Phase I	USA
	Aortic dissection (Multiple organ failure)	MSC-EVs	NCT04356300	N.A	Fujian Medical University
Skin	Dystrophic Epidermolysis Bullosa	MSC-EVs (AGLE 102)	NCT04173650	Phase I/IIA	N.C
	Skin ulcer	Plasma-derived EVs	NCT02565264	Phase I	Japan
	Chronic ulcer	Stem cell-conditioned media	NCT04134676	Phase I	Indonesia
	Wound healing	Platelet-EVs	NCT02565264	Phase I	Japan
Eye	Macular holes	MSC-EVs	NCT03437759	Phase I	China
	Dry eyes	UMSC-EVs	NCT04213248	Phase I and II	China
Pancreas	Diabetes Mellitus Type 1	UMSC-EVs	NCT02138331	Phase II and III	Egypt
	Pancreatic ductal adenocarcinoma	MSC-EVs loaded KrasG12D siRNA	NCT03608631	Phase I	USA

Information obtained from https://clinicaltrials.gov/ on 28 April 2021

BMMSC, bone marrow-drived mesenchymal stem cells; CM, conditioned medium; ADMSC, adipose tissue-derived MSC; PRP, platelet-rich plasma; PEP, purified exosome product; UMSC, umbilical mesenchymal stem cells

tissue repair and regeneration. The EV clinical studies in the field of regenerative medicine that are ongoing are summarized in Table 1.

In just a few years, several biotech companies have developed EV-based therapeutic agents from different cell sources, and have attempted to enhance the therapeutic potential of EVs using various strategies, including technology related to enhanced isolation efficiency, characterization, large-scale production, and loading cargo with a combination of other biomaterials. Commercial EV-based products for tissue repair and regeneration of other organs in human clinical settings have already been developed and registered (Table 2). Although EVs have shown potential as a new biological therapeutic agent in the field of regenerative medicine, and their effectiveness has been verified through in vivo and in vitro studies, the mechanisms by which the biological components of EVs promote tissue repair and regeneration remain unknown. However, once the relative contributions of specific molecules become clear, researchers will be able to enhance the

Table 2 Lists of companies developing EV-products for regenerative medicine

Company	EV-product	Target	Homepage
Codiak Biosciences (USA)	exoSTING™	Cancer	https://www.codiakbio.com/
	exoIL-12™	Neuronal disease	
	exoASO™-STAT6		
Evox Therapeutics (UK)	EVOX-101	Rare genetic disorder (Argininosuccinic aciduria, Citrullinemia type I, Phenylketonuria)	https://www.evoxtherapeutics.com/
	EVOX-102		
	EVOX-103		
Capricor Therapeutics (USA)	CAP-2003	Duchenne muscular dystrophy	https://capricor.com/
	Engineered EVs	COVID-19	
Aegle Therapeutics (USA)	AGLE-102	Epidermolysis bullosa	https://www.aegletherapeutics.com/index.html
		Burn	
ExoPharm (Australia)	Engineered EVs	Genetic diseases	https://exopharm.com/
		Neurodegenerative diseases	
		Viral infections	
		Cancer	
ReNeuron (UK)	CTX-derived Exosomes	Drug delivery	http://www.reneuron.com/
Anjarium Biosciences (Switzerland)	Hybridosomes® (lipid synthetic particles + EVs)	Cancer	http://www.anjarium.com/
		Rare genetic diseases	
Innovex Therapeutics (Spain)	Exosomes	Malaria	https://innovexther.com/
		COVID-19	
		porcine reproductive and respiratory syndrome virus (PRRSV)	
Carmine Therapeutics (USA)	REGENT®	Genetic diseases	https://www.carminetherapeutics.com/
Evora Biosciences (France)	EVOGEX	Digestive fistula	https://www.evorabio.com/
Vesigen Therapeutics (USA)	Engineered ARMMs((ARRDC1)	Neurologic diseases	https://www.vesigentx.com/programs/
		Ophthalmologic diseases	
		Cancer	
Exogenus Therapeutics (Portugal)	Exosomes	Skin diseases	
		Autoimmune diseases	
Aruna Bio (Greece)	AB126 (exosomes)	Neurodegenerative diseases	https://aruna-bio.webflow.io/
	AB127(siRNA)		
	AB128(protein)		
	AB129(mRNA)		
Organicell (USA)	Zofin	Musculoskeletal diseases	https://organicell.com/
	Pure X	Chronic obstructive pulmonary disease (COPD)	
		Cardiac diseases	
		Autoimmune diseases	
		Neurologic diseases	
		COVID-19	
MDimune (South Korea)	BioDrone®	Osteoarthritis	http://www.mdimune.com/
		COPD	
		Neurodegenerative diseases	
ILIAS Biologics (South Korea)	EXPLOR™	Inflammatory diseases	http://iliasbio.com/
	Exo-Target®	Metabolic genetic diseases	
		(Gaucher's Disease; GD2 and 3)	
OmniSpirant (Ireland)	Exosomes	Cystic fibrosis	https://www.omnispirant.com/
		COPD	

Table 2 continued

Company	EV-product	Target	Homepage
NeurExo (USA)	NXS-1001	Stroke	http://nxs.hartzcreative.com/
	NXS-1002	Mild Traumatic brain injury (TBI)	
	NXS-1003	Mild to Moderate TBI	
Creative Medical Technology Holdings (USA)	ImmCelz®	Stroke	https://creativemedicaltechnology.com/
Infusio (Germany)	Exosomes	Lyme disease	https://www.infusio.org/
		Chronic inflammation	
		Autoimmune diseases	
		Chronic degenerative diseases	
		Anti-aging therapies	
Exocel Bio (USA)	Exovex	Cellular rejuvenation	https://www.exocelbio.com/
ExoCoBio (South Korea)	ExoSCRT	Skin diseases	http://www.exomage.co.kr/index
	ExoBRID-E and Vexosome EVs+	Cellular rejuvenation	
	ASCE + ™		
	: Exomage		
	Celltweet		
Versatope Therapeutics (USA)	Recombinant Outer Membrane Vesicles	Allergy	https://www.versatope.com/
		Vaccine development	
		Infectious diseases	
PureTech Health (USA)	Orasome™	Rheumatoid arthritis,	https://www.puretechhealth.com/
		Diabetes,	
		Autoimmune diseases cancer	

therapeutic potential of EVs via biochemical or genetic engineering for disease- and organ-specific repair and regeneration.

The special issue "Current progress in extracellular vesicles in stem cells and tissue regeneration" was enthusiastically released by the Editorial Board of *Tissue Engineering and Regenerative Medicine* to identify unresolved issues and report on cutting-edge developments in tissue engineering and regenerative medicine. As reviewed in this special issue, advances in the isolation and characterization of EVs, along with their intrinsic capacity, clearly opens new avenues for tissue repair and regeneration in humans. We would like to thank all of the contributing authors of the papers collected in this special issue and hope that the readers will both enjoy and be inspired by this emerging and state-of-the-art research topic.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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