

Emerging Roles for Extracellular Vesicles in Tissue Engineering and Regenerative Medicine

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Extracellular vesicles (EVs)—comprising a heterogeneous population of cell-derived lipid vesicles including exosomes, microvesicles, and others—have recently emerged as both mediators of intercellular information transfer in numerous biological systems and vehicles for drug delivery. In both roles, EVs have immense potential to impact tissue engineering and regenerative medicine applications. For example, the therapeutic effects of several progenitor and stem cell-based therapies have been attributed primarily to EVs secreted by these cells, and EVs have been recently reported to play direct roles in injury-induced tissue regeneration processes in multiple physiological systems. In addition, EVs have been utilized for targeted drug delivery in regenerative applications and possess unique potential to be harnessed as patient-derived drug delivery vehicles for personalized medicine. This review discusses EVs in the context of tissue repair and regeneration, including their utilization as drug carriers and their crucial role in cell-based therapies. Furthermore, the article highlights the growing need for bioengineers to understand, consider, and ultimately design and specifically control the activity of EVs to maximize the efficacy of tissue engineering and regenerative therapies.

Introduction

TISSUE ENGINEERING AND REGENERATIVE medicine are strongly associated with the controlled application of cells for therapy, often in combination with biocompatible materials. Technological advances such as three-dimensional printing have opened up exciting possibilities in these fields; however, development of enhanced understanding of cellular function and especially intercellular communication also offers transformational potential for improving tissue engineering strategies. Additionally, tissue repair and regeneration through drug therapies—either as part of an integrated therapeutic approach involving cells or as an alternative to cellular therapies—remains a tantalizing option given potential advantages over cells with regard to reproducibility, scalability, quality control, and regulatory considerations.

One significant recent development in the understanding of intercellular communication is the appreciation of the role of extracellular vesicles (EVs).¹ Once considered to be mere cellular debris, EVs—composed of exosomes, microvesicles, and other heterogeneous vesicles typically sized in the 30–2000 nm range that are shed from cells either by blebbing from the plasma membrane or by the endolysosomal pathway—are increasingly being recognized as vital mediators of information transfer between cells (we refer the reader to several excellent recent reviews covering the biogenesis of

EVs and their role in cell–cell communication^{2–4}). The authors note that many published studies describe activities or properties of specific EVs such as exosomes or microvesicles; however, due to variability in EV isolation and characterization methods as well as to the continuing development of knowledge of EV biogenesis, identification of these specific vesicle populations is problematic and terms are often used interchangeably. Thus, in this review, we will refer to all vesicle populations as EVs. Importantly, EVs have been demonstrated to be vital components of cell-based therapies; in some cases, the therapeutic efficacies of cell-based interventions are almost entirely attributable to paracrine effects through EVs.^{5,6} Yet, the literature reflects that the role of EVs in many cell-based therapies has yet to become a primary design consideration in tissue engineering, as issues such as the potential effects of culture conditions and cell–biomaterial interactions on EV biogenesis and content are scarcely reported.

Interestingly, EVs also have significant potential as drug and gene carriers for regenerative therapeutic applications. For example, seminal work by Wood and colleagues demonstrated that EVs can be loaded with a desired genetic cargo and targeted to a specific cell type by straightforward methods.⁷ Additional studies have shown that EVs are potentially versatile drug carriers that could be applied in wide-ranging applications toward regeneration.^{8–10} However, the full potential of EVs as

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mediators of tissue repair and regeneration by drug and gene delivery is still unknown.

Overall, EVs have substantial promise to play important roles in tissue engineering and regenerative therapies. Despite this promise, many questions remain to be answered before EVs are likely to be a significant consideration of a vast majority of scientists and engineers at the design stage of tissue engineering and regenerative therapeutic approaches. In this article, we review reports of EVs related to tissue engineering and regenerative therapies, with a focus on the most recent literature, and attempt to summarize current knowledge in this area. Specifically, the roles of EVs in cell therapies and as drug and gene carriers are highlighted and integrated into a discussion of what additional knowledge might lead to EVs becoming more broadly considered as crucial players in the fields of tissue engineering and regenerative medicine.

EVs as Paracrine Mediators of Cell-Based Therapeutic Regeneration

EVs are released from numerous cell types and can transfer information—including mRNA, miRNA, and proteins^{1,2,4} as well as DNA^{11,12}—from cell to cell. In the context of cell therapy, EVs can act in a paracrine manner to alter the phenotypes of recipient cells, potentially promoting repair and regeneration. The therapeutic benefit of EVs in cell-based therapies has been observed in several different physiological systems that are of interest in tissue engineering and regenerative medicine, and cell source is a critical factor, with EVs derived from stem or progenitor cells—and especially from mesenchymal stromal cells (MSCs)^{6,13,14}—having shown promising regenerative effects in several of these systems.

Nervous system

Nerve repair and regeneration has long been a significant goal in tissue engineering and regeneration.^{15–17} Most cell types within the nervous system (NS) are thought to release EVs, and the role of exosomes—a particular subset of EVs—in information transfer in the NS is well established.^{18–20} Pioneering work (discussed in more detail later in this article) in harnessing EVs for drug delivery was conducted in the NS,⁷ and the roles of exosomes and other EVs in NS development, normal function, and pathophysiology are expertly reviewed elsewhere.²¹ With regard to NS regenerative therapies, several recent reports have revealed the therapeutic potential of EVs. Xin *et al.* showed that EVs emanating from MSCs transfer miR-133b to neural cells, inducing neurite outgrowth and enhancing functional recovery from stroke in a rodent model.^{22–24} Kraig and colleagues reported the utility of dendritic cell-derived EVs in inducing remyelination of damaged nerve fibers, which could have significant implications for the treatment of multiple sclerosis and other NS pathophysiologicals.²⁵ In this study, interferon- γ stimulation was used to promote expression and EV packaging of miRNAs associated with myelin production, enhancing the regenerative nature of the EVs and demonstrating how EV cargo is susceptible to change based on source cell microenvironmental conditions.²⁵ Schwann cell-derived EVs have also been shown to induce regeneration in the NS.²⁶ Court and colleagues discovered that Schwann cells, well-known inducers of axonal regeneration, utilize EVs as part of their overall regulation

and stimulation of axonal growth after injury. This study further demonstrates the capability of Schwann cell-derived EVs to enhance axonal regeneration after sciatic nerve injury *in vivo*,²⁶ providing direct support for the potential application of NS cell-derived EVs for peripheral NS regenerative therapies.

Importantly, the study by Court and colleagues also suggests that EVs should be considered in peripheral NS tissue engineering strategies (e.g., for spinal cord repair). This suggestion is buttressed by the finding of Fruhbeis *et al.* that EVs play a critical role in oligodendrocyte–neuron communication toward promoting axonal integrity.²⁰ Thus, the maintenance of appropriate EV biogenesis and signaling in cell-based tissue engineering approaches for treatment of NS injury may be a crucial factor in the potential long-term success of any intervention.

Vascular system

Therapeutic control of vascularization has long been sought as a critical enabling technology for complex tissue engineering strategies, and numerous drug delivery and cell transplantation strategies have been developed toward this goal.^{27–34} Recent studies have focused on exploring the roles of EVs in autocrine and paracrine signaling during vascular development and maturation and as potential drug delivery vehicles for therapeutic vascularization. A study by van Balkom *et al.* showed that endothelial cells (ECs) secrete miR-214-rich EVs, which promote EC migration and angiogenesis *in vitro* and *in vivo* through repression of ataxia telangiectasia mutated (ATM) expression in recipient cells,³⁵ revealing a crucial role for EVs in EC autocrine signaling. Dimmeler and colleagues reported that miRNA transfer from ECs to smooth muscle cells (SMCs) through EVs could induce an atheroprotective SMC phenotype,³⁶ indicative of a paracrine signaling role for EVs in the vascular system and establishing the potential for EC-derived EVs to be employed as therapeutic drug/gene carriers for treatment of vascular and cardiovascular diseases. EVs from endothelial progenitor cells (EPCs) also have physiological importance as demonstrated by Deregibus *et al.*, who showed that EPC-derived EVs activate an angiogenic program in ECs through horizontal transfer of mRNA.³⁷ Furthermore, Cantaluppi *et al.* showed that EPC-derived EVs carrying proangiogenic miR-126 and miR-296 sustain islet vascularization and maintain β -cell function by transfer of miRNAs to islet endothelium, resulting in activation of PI3K-Akt and eNOS signaling pathways.³⁸ Also, as in other systems, MSC-derived EVs can therapeutically impact the vascular system. Salomon and colleagues showed that placental MSC-derived EVs induce human placental microvascular EC migration and tube formation,³⁹ and Xin *et al.* demonstrated that, in addition to improving overall functional recovery, the administration of MSC-derived EVs to rats after stroke enhances angiogenesis.²⁴ Additionally, Losordo and colleagues showed that the proangiogenic paracrine activity associated with human CD34⁺ stem cells can be almost entirely attributed to EVs secreted from these cells.⁵ In total, these studies solidify the importance of EVs in physiological vascular signaling and demonstrate the potential of EVs for therapeutic delivery to vascular cells. Both these roles merit consideration at the design stage of tissue engineering and regenerative medicine approaches involving vascularization.

Cardiovascular system

Heart disease and dysfunction remain leading causes of mortality and morbidity worldwide, and the innate regenerative capacity of the heart is inadequate to compensate for any significant decrease of function due to damage or death of cardiomyocytes^{40,41}; thus, cardiac regeneration is currently a high priority goal within the fields of tissue engineering and regenerative medicine.^{42,43} Recent evidence suggests a potential role for EVs in cardiovascular homeostasis, and there have already been several demonstrations of the therapeutic efficacy of EVs on the cardiovascular system. Sahoo and Losordo demonstrated that both mouse and human cardiomyocytes are able to produce EVs under normal and ischemic conditions,⁴⁴ and Barile *et al.* provided evidence, in mice, that cardiovascular progenitor cells secrete EVs.⁴⁵ Further understanding of any differences in the distribution, function, and fate of secreted EVs in the cardiovascular system will undoubtedly enhance the design of multicellular tissue engineering strategies and provide insight into cell-based cardiac regeneration approaches.

In animal models, the uptake of EVs in the heart is supported by the therapeutic effects of MSC-derived EVs in reducing myocardial/ischemia reperfusion injury,⁶ enhancing blood flow recovery following acute myocardial infarction⁴⁶ and reducing cardiac fibrosis in infarcted hearts.⁴⁷ The proangiogenic effects of EVs also contribute to their cardiovascular therapeutic efficacy. Vrijnsen *et al.* showed that cardiomyocyte progenitor cell-derived EVs stimulate EC migration.⁴⁸ Furthermore, Mackie and colleagues engineered CD34⁺ stem cells to release EVs containing sonic hedgehog, a proangiogenic factor. Injection of these modified cells to the border zone of mice after myocardial infarction resulted in reduced infarct size, increased capillary density, and improved functional recovery.⁴⁹ Thus, advances in the application and engineering of EVs as therapeutic delivery vehicles may lead to new possibilities for cardiovascular therapies.

Reproductive system

Tissue engineering has already begun to deliver on the promise of repairing or regenerating organs or tissues in the reproductive system, as exemplified by a recent report of successful functional engineering of vaginal tissues in human patients,⁵⁰ and additional potential applications for reproductive tissue engineering and regeneration include male reproductive organs as well as the uterus and cervix.⁵¹ The direct regenerative potential of EVs emanating from reproductive tissues is unclear, although evidence has begun to emerge that EVs secreted by (uterine) endometrial epithelial cells are critical for successful embryo implantation in pregnancy⁵² and that EV-mediated miRNA transfer within the ovarian follicle is crucial for healthy oocyte development.⁵³ In addition, placental EVs were previously identified as vital immunoregulatory agents in pregnancy by Taylor, Gercel-Taylor and colleagues.^{54,55} In the male reproductive system, EVs secreted by the epididymis and prostate function to protect sperm and promote its maturation,⁵⁶ enhancing reproductive potential. All these findings suggest that the role of EVs should be considered in the design of tissue engineering or regenerative medicine approaches involving reproductive tissues or organs. For example, Campbell *et al.* reported a strategy for growth of uterine tissue in the peritoneal cavity of

host animals before transplantation to the appropriate anatomical location.⁵⁷ In this type of *in vivo* bioreactor approach to tissue engineering, it may be critical to examine what effect the generation of tissue in nonorthotopic locations has on EV biogenesis and content and to understand what information may be transferred to these tissues during their pre-transplantation maturation by EVs. Additionally, Ulrich *et al.* recently reported the use of MSCs derived from human endometrium as a component of a tissue engineering strategy to address pelvic organ prolapse.⁵⁸ It is likely that EVs play at least some role in the effects of the endometrial MSCs in this system, and thus, it may be of interest to compare the rates of biogenesis and, especially, the cargos of EVs derived from endometrial MSCs to those of other MSC-derived EVs.

Hepatic system

Although the liver has significant intrinsic regenerative capacity, liver failure is still a prevalent health problem worldwide and tissue engineering approaches are being sought to ease the shortage of donor organs. Many such strategies involve hepatocyte transplantation,^{59,60} and recent reports indicate a potential role for hepatocyte-derived EVs in liver regeneration. Nojima *et al.* demonstrated a dose-dependent increase in hepatocyte proliferation when ischemia/reperfusion (I/R)-injured mice were treated with hepatocyte-derived EVs.⁶¹ Likewise, Herrera *et al.* demonstrated the therapeutic properties of human liver stem cell-derived EVs to accelerate liver regeneration in a 70% hepatectomized rat model.⁶² In this study, RNA transfer from EVs into target hepatocytes was mediated by the presence of $\alpha 4$ -integrin adhesion molecules on EV surfaces,⁶² indicative of specificity for this mode of intercellular information transfer. Additionally, Royo *et al.* demonstrated that EVs derived from primary hepatocytes activated hepatic stellate cells by means of RNA transfer.⁶³ In this study, hepatic stellate cells treated with EVs derived from primary hepatocytes showed increased expression of nitric oxide synthase 2 (NOS2), an enzyme characteristic of stellate cell activation.⁶³ RNase-pretreated hepatocyte EVs did not evince increased NOS2 expression,⁶³ highlighting the essential role of EV-mediated RNA transfer in hepatic stellate cell activation, which is known to occur during the early stages of liver regeneration.⁶⁴ Furthermore, proteomic analysis of EVs from animals following experimentally induced liver injury revealed that the liver-specific enzyme S-adenosylmethionine synthetase 1, essential for liver regeneration, was present in hepatocyte-derived EVs.⁶⁵

All the studies referenced above point toward the need for consideration of EVs in liver tissue engineering applications, and some work has already been done to illuminate the mechanisms involved in EV biogenesis and secretion in hepatocytes. Nojima *et al.* recently demonstrated that CXCR1 and CXCR2, which have previously been shown to play a role in the recovery and regeneration of the liver after I/R injury, influence EV secretion by hepatocytes.⁶¹ This study revealed that CXCR2^{-/-} hepatocytes produced EVs in similar quantities to those of wild-type hepatocytes, whereas CXCR1^{-/-} hepatocytes produced considerably fewer EVs.⁶¹ Expansion of this genre of research would enable more thorough understanding and consideration of the role of EVs in liver regeneration, potentially leading to more effective liver regeneration and tissue engineering therapies.

Respiratory system

Given the diversity of cell types within the lungs, the establishment of appropriate intercellular communication is likely to be a key feature of any effective lung regeneration or tissue engineering approach. The importance of EVs in cell-cell communication¹ suggests consideration of their effects, and indeed, EVs derived from MSCs have been shown to exert a pleiotropic protective effect on the lung resulting in the inhibition of pulmonary hypertension.⁶⁶ It has also been established that EVs captured from fluid within the lungs have different cargoes in patients with lung diseases, such as asthma and sarcoidosis, compared with those in patients with typical lung function.^{67–69} Furthermore, EVs isolated from bronchoalveolar fluid of mice tolerized to an olive pollen antigen were shown to induce tolerance and protection in naive mice following intranasal administration,⁷⁰ indicative that information from EVs collected in the lung can be processed to produce a physiological response. Most interestingly, Quesenberry and colleagues showed that lung-derived EVs stably reprogram marrow cells toward a pulmonary epithelial phenotype.^{71,72} Given the strong evidence of significant roles for EVs in lung physiology, EVs should be considered when designing strategies that build on the exciting initial efforts in lung tissue engineering.^{73,74}

Renal system

EVs in the renal system are often discussed in the context of their potential use as biomarkers that can be isolated from urine.^{75,76} Also, as with many of the other physiological systems mentioned, MSC-derived EVs have been shown to have therapeutic effects on the kidneys,^{38,77–79} indicating that renal epithelial cells are capable of taking up EVs and processing information that results in phenotypic changes. In addition, pioneering work by Knepper and colleagues revealed that renal epithelial cells secrete EVs,⁸⁰ providing a basis for the consideration of the role of EVs in renal system regenerative and tissue engineering approaches, although the role of EVs in intercellular communication between renal epithelial cells is not well defined. It has been shown, however, that renal fibrosis can lead to altered miR-29c content in

urinary EVs,⁸¹ further evidence that EVs play a significant role in renal epithelial signaling and function and should be considered by tissue engineers employing differentiated or embryonic epithelial cell-based regenerative approaches to produce kidneys^{82,83} or other aspects of the renal (or urinary) system.^{84,85}

Summary

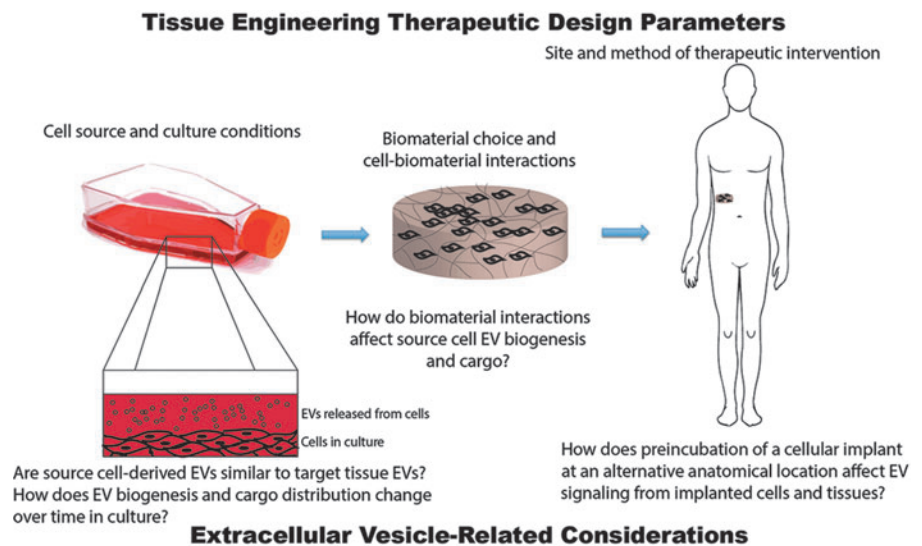
The roles of EVs in tissue homeostasis and regeneration in numerous physiological systems are only beginning to be fully understood, yet the preponderance of evidence reported to date indicates that these roles are likely to be significant in at least some tissues and organs. Beyond what has been discussed above, the development of engineered tissues as both model systems for drug development^{86,87} or for study of complex biology *in vitro* would benefit from consideration of the roles of EVs. This is especially relevant for tissue engineering models of cancer,⁸⁸ where roles for EVs in tumor growth and metastasis are well described.^{89–93}

Overall, the consideration of EVs in the design stage of tissue engineering and regeneration approaches is warranted. For example, the choice of cell source for cell-based therapies might be impacted by knowledge of how EV signaling in the chosen cells relates to what is known about physiological EV signaling in the target organ or tissue. However, for such consideration to ever be undertaken generally, more fundamental studies of EV biology in the context of tissue engineering and regenerative medicine are needed. How are EV biogenesis and cargo content affected by cell culture conditions or cell–biomaterial interactions? Do induced pluripotent stem cells recapitulate EV biogenesis of the natural cells into which they have been differentiated? These and other questions await answers that have the potential to have a substantial impact on the practice of tissue engineering and regenerative medicine in the future (Fig. 1).

EVs as Therapeutic Delivery Vehicles for Tissue Regeneration

The emerging appreciation for the critical roles of EVs in mediating therapeutic effects of cell-based interventions not

FIG. 1. Consideration of extracellular vesicles (EVs) in the design stage of tissue engineering approaches. Tissue engineers consider many parameters in designing therapeutic approaches, including cell source and culture conditions, biomaterial–cell interactions, and the site and method of therapeutic intervention (e.g., injection vs. implantation, use of an alternative anatomical location as an *in vivo* bioreactor). Consideration of each of these parameters could be affected by further knowledge of how EV biogenesis and cargo may change based on the choices made at each step of the tissue engineering design process. Color images available online at www.liebertpub.com/teb



only highlights the need for consideration of EV biology in tissue engineering, it also reveals potential applications for EVs as natural delivery vehicles for biologics (e.g., mRNA, miRNA, DNA, and proteins) for tissue regeneration. As alternatives to cellular therapies, EVs have advantages with regard to safety and regulatory concerns—EVs do not have the replicative capacity of cells—and are also less complex and more amenable to quality control and, potentially, scale-up when compared to cells. Furthermore, EVs have the potential to be used generically as drug carriers and could be uniquely employed in personalized medicine if obtained from a patient's own cells. Thus, in addition to increased knowledge of EV biology, the fields of tissue engineering and regenerative medicine may benefit from advances in EV-related biotechnology.

Unmodified EVs as alternatives to cell therapies

Perhaps, the most straightforward utilization of EVs in therapeutic delivery involves the harvesting and administration of unmodified EVs containing their native cargo as surrogates in place of the source cells that produce the EVs. EVs are most commonly isolated from cell cultures by differential centrifugation, immunoprecipitation, or chromatographic methods,^{94,95} and it should be noted that current methods may not efficiently separate exosomes from microvesicles and/or other EVs; thus, reports of activities of specific EV populations should be taken with appropriate context. The potential efficacy of using unmodified EVs as surrogates is clearly dependent on cell source, and as highlighted earlier in this review, MSCs have proven to be a viable source of EVs for a variety of therapeutic interventions.^{8,14} Lim and colleagues have reported the development of a potentially infinite supply of MSC-derived EVs through oncogenic immortalization of human embryonic stem cell (ESC)-derived MSCs,⁹⁶ an advance that may broaden the therapeutic applicability of MSC-derived EVs. However, data from our group suggest that cell immortalization strategies must be considered on a

case-by-case basis, as EVs isolated from retrovirally telomerized ECs⁹⁷ display vastly different RNA and protein profiles than those from native ECs derived from human umbilical vein and human dermal microvasculature (T.N.L. and S.M.J., unpublished). Nevertheless, the numerous reports of therapeutic effects of MSC-derived EVs demand their consideration as alternatives to MSC-based cellular regeneration strategies.

In addition to MSCs, stem and progenitor cells have been identified as producers of EVs with native therapeutic potential. This is not surprising, given that paracrine effects of implanted adult stem cells, like those of MSCs,⁹⁸ are thought to account for the majority of their therapeutic impact in numerous studies.^{99,100} Working with murine ESCs, Rajczak and colleagues showed that EVs derived from these cells induced enhanced survival and improved expansion of murine hematopoietic progenitor cells.¹⁰¹ As noted earlier, human CD34⁺ stem cell-derived EVs mediate the therapeutic effects of these cells,³⁷ and both cardiomyocytes and EPCs produce EVs with biological activity that could be utilized therapeutically.^{38,48,102} It seems likely that, given the convergence of the increased understandings of the roles of paracrine factors in cell therapies and of EVs in paracrine effects of cells, stem cell-derived EVs will continue to emerge as viable alternatives to cellular regenerative therapies for many applications.

Engineering EVs for therapeutic delivery

Although exploitation of the native capacity of EVs to mediate therapeutic effects is a promising avenue, the therapeutic efficacy and versatility of native EVs is limited by their native cargo and targeting capacity. Engineering of EVs for therapeutic delivery offers the potential to load non-native cargo—including nonbiologic molecules such as small molecule drugs (e.g., doxorubicin⁹)—and to add non-native abluminal moieties to affect pharmacokinetics and biodistribution. These possibilities, combined with the

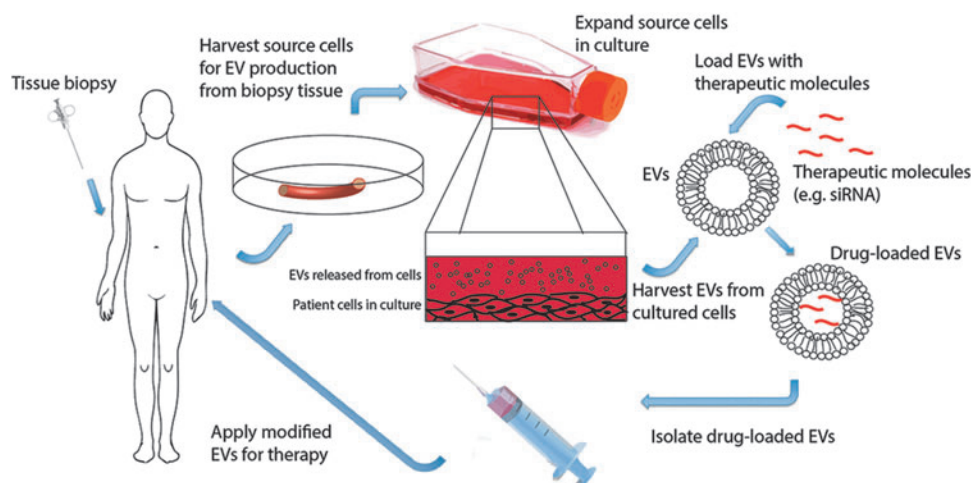


FIG. 2. Engineering of EVs for use as therapeutic delivery vehicles in personalized medicine. EVs offer unique potential to be employed as delivery vehicles in personalized medicine. EVs could be obtained from cells harvested from biopsy tissue, loaded with a therapeutic molecule, and injected back into the patient. Such an approach would enable protection of labile therapeutic biologics while also avoiding the immunogenicity inherently associated with synthetic delivery systems. Color images available online at www.liebertpub.com/teb

unique nature of EVs that allows for their isolation from a patient's own cells for application in a personalized medicine approach as self-derived drug delivery vehicles, endow EVs with the potential to revolutionize drug delivery (Fig. 2).

A template for EV engineering was established in pioneering work by Wood and colleagues, who harvested EVs from dendritic cells of mice for ultimate injection into recipients with the same major histocompatibility complex haplotype.⁷ Before EV isolation, cells were transfected with plasmids encoding a fusion of either a muscle-specific or central NS-specific targeting peptide sequence, and Lamp2b, a protein known to associate with EV membranes, allowing for the targeting peptide sequences to be presented abuminally on EVs.⁷ This strategy was proven effective for multiple targeting peptides and has been expanded by others to target EGFR-expressing cancer cells for therapeutic delivery.¹⁰³ Once isolated, the EVs were loaded with therapeutic siRNA molecules by electroporation before being administered to mice by intravenous injection.⁷ The result of this intervention was brain-specific knockdown of *BACE1*,⁷ a protease involved in Alzheimer's disease, serving as proof of concept that EV engineering has revolutionary potential for therapeutic delivery.

Excitingly, there are many paths forward to expand upon these initial groundbreaking findings. Gould and colleagues have elucidated mechanisms of protein targeting to EVs^{104–106} that could be utilized for controlled loading of protein cargo and/or expression of targeting ligands on EVs. Loading of EVs with nonbiologic therapeutic cargo has been reported,^{9,10,107} expanding the horizons for the types of therapeutic interventions that are possible with EVs. In addition to the myriad mammalian cell types that can be used as source cells for EVs, alternative sources of EVs such as fruit have been identified,^{108,109} which could open new doors for the scalable production of EVs for therapy.

Summary

The developments listed above, combined with the inherent advantages of EVs over synthetic drug delivery systems with regard to immunogenicity and ability to deliver cargo intracellularly and across biological barriers,¹¹⁰ spark great optimism for the potential of EVs in therapeutic biologic delivery. However, many challenges remain before widespread adoption of native or engineered EVs for therapeutic delivery applications. For example, standardization of EV isolation and characterization methods is needed to facilitate universality and reproducibility of findings,¹¹¹ and large-scale production methods for therapeutic EVs must be developed. Additionally, better definition of EV pharmaceutical parameters, such as loading capacity and target ligand surface density, would help to clarify their therapeutic potential. Improved understanding of the biodistribution and trafficking of native EVs and how they are affected by cell source could enable more effective targeted therapeutic delivery. It is also important to consider how native cargo content is affected by cell source, cell culture conditions, and mechanical forces, and genomic and proteomic analyses should be applied to bring clarity to these issues. Finally, the drug release characteristics and tissue targeting and uptake efficiency of EVs must be directly compared to polymeric nanoparticle and liposomal delivery systems for specific cargoes to determine the true relevance of EVs as drug carriers. Resolution of these

considerations along with continued development of EVs as therapeutic delivery vehicles and continued advances in understanding of EV biology will likely lead to new therapeutic opportunities involving EVs in tissue engineering and regenerative therapies in the future.

Conclusion

EVs have ubiquitous effects in biology and should be considered at the design stage of regenerative therapies and tissue engineering approaches. Additionally, EVs may be useful as delivery vehicles for biologics and as alternatives to cell-based tissue engineering or regenerative interventions. Overall, EVs should be recognized as key players in tissue engineering and regenerative medicine in a general sense.

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Disclosure Statement

The authors declare no conflicts of interest.

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