Therapeutic potential of mesenchymal stem cells and their secreted extracellular vesicles in thoracic aortic aneurysm disease

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Supplemental material is available online.

Thoracic aortic aneurysm (TAA) represents a major cause of morbidity and mortality and continues to be a difficult management problem for cardiovascular surgeons. Each year in the United States, nearly 10,000 people die from aortic aneurysms, and an additional 16,000 die from complications associated with aortic disease. Aortic aneurysm is the 17th-leading cause of death for those older than 65 years of age.1,E1 Despite advancements in our understanding of the pathobiology of TAA, no effective medical therapeutic exists.

Mesenchymal stem cells (MSCs) have shown efficacy in cardiovascular disease, and safe use in clinical applications has been verified. MSCs arrest pathologic extracellular matrix (ECM) remodeling, suggesting a therapeutic potential in TAA. Preclinical studies show successful delivery of MSCs to abdominal aortic tissue by direct periadventitial application and intravenous (IV) administration; treatment efficacy in TAA, however, has yet to be fully explored. Once delivered to a recipient with pathologic vascular damage, MSCs traffic to the site of injury.2 Homing occurs in response to gradients of cytokines released by aortic cells. Importantly, elevated cytokines, such as the transforming growth factor-β, are secreted from TAA tissue and promote MSC homing in vivo.3,E2 (Figure 1).

MSCs consistently improved outcome measures in preclinical studies. Results remained robust despite differences in method of administration, comorbidities, dosage, or species of MSC origin.3,E3 There are approximately 1200 ongoing clinical trials investigating the medical utility of MSCs. In addition to highlighting safe use in humans, these trials demonstrate that MSCs can be effective in treating disparate pathologies and bodily injuries ranging from spinal cord trauma and liver failure to myocardial infarction.4,5,E2,E4 More recently, MSCs have been identified as mediators of vascular ECM remodeling through multitargeted suppression of multiple proteolytic enzymes and cytokines, suggesting a therapeutic potential in TAA.

A mechanism by which MSCs suppress proteolytic activity and cytokine release is via transfer of nucleic acids. One of the major nucleic acid subtypes transferred is micro-RNAs. MicroRNAs have the unique ability to target many different mRNAs and suppression translation. Thus, MSCs have the unique ability to alter complex pathways, such as cellular phenotype transformation, and ECM remodeling.

Therapeutic benefits of MSCs are not always contingent upon engraftment or differentiation at the site of injury.4,5 Therapeutic effects have been attributed to secretion of
key mediators of ECM remodeling packaged in lipid bilayer vesicles known as extracellular vesicles (EVs). EVs carry a variety of cargo that can be taken up by other cells, eliciting a variety of phenotypic responses.

The term MSC has become the preferred acronym to describe a population of multipotent stem/progenitor cells, commonly referred to as mesenchymal stem cells, multipotential stromal cells, mesenchymal stromal cells, or mesenchymal progenitor cells. MSCs may be isolated autogenously or from a donor; they can then be expanded, ex vivo, and injected IV into a recipient.

There are several adult tissue sources for MSCs. These include bone marrow, umbilical cord Wharton’s jelly, and adipose tissue. MSCs of different tissue origins can confer substantially different therapeutic effects in ameliorating pathologies. Bone marrow–derived MSCs are the most frequently used type in clinical settings; however, there are limited data available concerning bone marrow–derived MSCs in TAA (other investigators have used human umbilical MSCs in mice and xenografting). Of note, MSCs can be harvested directly from long bone marrow, making them fairly easy to obtain from mice.

MSCs may be isolated autogenously or from a donor, then can be expanded ex vivo, modified by transfection, and injected IV into a recipient. MSCs traffic, or home, to the site of injury. Homing occurs in response to gradients of cytokine mediators released by pathologic tissues. Importantly, elevated transforming growth factor-β, as seen in aneurysm tissue, has been demonstrated to promote homing of MSCs in vivo. A recent single-cell transcriptome analysis revealed that MSCs are present in clinical TAA specimens, further demonstrating homing potential. Alternatively, MSCs and MSC EVs have been successfully delivered to and incorporated in vascular tissue in vivo by direct periadventitial application.

**SUMMARY OF PRECLINICAL INVESTIGATIONS**

Multiple preclinical studies have investigated therapeutic application in abdominal aortic aneurysm (AAA). Although the pathologies of AAA and TAA are very different, the many persistent similarities in pathologic progression are germane, as they imply potential for overarching therapeutic efficacy despite disparate patho-biogeneses. A meta-analysis of the PubMed database, performed in 2022, identified 18 studies concerning MSCs, AAA, and therapeutic effectiveness: suppressing aortic diameter enlargement, reducing elastin degradation, and modulating local immuno-inflammatory reactions. Even though methodology varied widely between studies (for example, source of MSCs and number delivered), results consistently...
demonstrated therapeutic efficacy in AAA. Taken together, these exciting data warrant further investigation.

IV injection of MSCs had therapeutic effects in both elastase-induced and AngII infused apolipoprotein-E knockout mouse models of AAA.11,12 MSC delivery consistently resulted in smaller aortic dilation and reduced elastin degradation in both models. Although temporal effects remain unknown, attenuation of dilation in 2 separate models of spontaneous abdominal aneurysm strengthens the possibility for therapeutic potential in TAA.

Investigations revealed that therapeutic efficacy is to some extent sex-dependent: when stem cells taken from a male or female mouse were injected, it resulted in different outcomes.13 That is, female-derived MSCs were more effective when administered to male mice; male-derived MSCs were less effective therapeutically when given to another male mouse. These data underscore the importance of sex as a biological variable.

In another study of AAA, researchers delivered MSCs alone and MSC-conditioned culture media to mice via IV administration.13 Their findings were somewhat surprising in that therapeutic effectiveness remained roughly the same in both cases. This discovery suggests that components in the culture media are responsible for therapeutic efficacy, not simply the MSCs themselves. The logical supposition, then, is that MSC trophic secretions are key—specifically, the MSCs secreted EVs, a finding consistent with the aforementioned study. It postulated that therapeutic effectiveness depends upon the cargo they carry, in particular microRNAs, in this case miR-147.14

Histologic investigations identified a significant increase in staining for MSC-specific markers in aneurysm tissue when compared with nonaneurysmal, normal aortic tissues.15 In addition, a more recent analysis of the cellular composition of the ascending thoracic aorta identified the presence of MSCs in clinical TAA specimens.16 Combined, these findings suggest MSCs have the capacity to home to aneurysm tissues.

In 2021, Hawkins and colleagues7 applied MSCs to aneurysms on the descending portion of the thoracic aorta. Using an elastase model of TAA, IV administration of umbilical-derived MSCs was sufficient in attenuation of aortic inflammatory cell infiltration, elastin degradation, and aortic diameter. An interesting finding of the study was the observation of modified microRNA profiles in thoracic aortic tissues following IV MSC delivery. With elastase treatment, 53 microRNAs were differentially expressed: 25 microRNAs were found up-regulated and 28 microRNAs down-regulated. However, following elastase treatment and IV MSC delivery, 43 microRNAs were differentially expressed: 40 miRNAs were found up-regulated and only 3 microRNAs were down-regulated. Interpretation was limited in that data could not actually show that the MSCs or MSC EVs were responsible for transferring miRNA to the tissue—only that most miRNAs went up in the tissue. The fact that so many microRNAs went up following MSC administration suggests direct transfer.

**TECHNICAL CHALLENGES ASSOCIATED WITH MSCs**

Studies show that MSCs are a promising potential therapeutic for TAA. However, current investigations are too various, using different methods of isolation, expansion, and approaches in characterization. In order to realize MSCs’ therapeutic potential, much remains to be done, especially normalizing techniques, standardizing protocols, and ensuring transparency in reporting.

We recognize that investigators report studies of MSCs using different methods of isolation and expansion, as well as different approaches to characterizing the cells (a summarized table of preclinical studies may be found in Li and colleagues10). Thus, it is increasingly difficult to compare study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal criterion to define human MSCs.17 First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express or not express several specific surface molecules. Third, MSCs must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro. The criteria for using human MSCs in therapeutic investigations are clearly defined; there are, however, no such criteria establishing accepted use of murine MSCs in preclinical studies.

Methods of isolating MSCs from bone marrow vary widely and include antibody-based cell sorting,7 low- and high-density culture techniques,17,18 positive/negative selection method,19 and an enzymatic digestion approach.20 Alternatively, isolating murine MSCs from long bone marrow using the frequent media change method is efficient and meets the aforementioned criteria.19

Precisely “optimal” culture conditions are still a matter of debate. Nevertheless, a detailed comparative analysis of culture conditions identified Dulbecco’s Modified Eagle’s Medium/Nutrient Ham’s Mixture F-12 as the best basal medium in which to perform prolonged MSC cultures, as they retain their ability to differentiate.20 Pal and colleagues20 showed that when cultured in Dulbecco’s Modified Eagle’s Medium/Nutrient Ham’s Mixture F-12, MSCs maintain morphology, population doubling time, and immunophenotype for more than 25 passages, demonstrating MSCs remain intact in culture long enough for up-scaling to reach amounts sufficient for transplantation.

Before being used in experiments, MSCs must be validated by flow cytometry. A murine model necessitates murine-specific surface markers for cell-type identification. Expert consensus dictates that identifying murine MSCs must be positive for CD29, CD44, CD105, and Sca-1 and
negative for CD34 and CD45 surface markers. The literature also includes alternative surface markers such as CD73 and CD80 positive and CD11b, CD117, CD31, CD86, CD90, CD105, and major histocompatibility complex class II negative. However, like MSCs, MSC EVs are uniquely challenging with which to work.

A search of ClinicalTrial.gov reveals 15 clinical trials involving MSC EVs, but none have been completed as of this writing. Although immunogenicity is low and safe delivery to humans has been established, size and amount often make them difficult to obtain as relatively pure preparations. Moreover, they are difficult to characterize properly. Accordingly, studies involving MSC EVs must adhere to the minimum criteria set forth by the Minimal Information for Studies of Extracellular Vesicles guidelines.

Many different EV subpopulations are found in the literature, and they are signified by varying nomenclature. These variations can be attributed to the subpopulations’ disparate cellular origin, biogenesis, function, size, cargo, and membrane markers. EVs are divided primarily into 2 groups: exosomes and microvesicles. Exosomes are typically between 30 and 120 nm and are released from multivesicular endosomes following fusion from the plasma membrane. Microvesicles are between 50 nm and 1 μm. Extremely heterogeneous in function and size, they are shed into the extracellular milieu by budding of the cell membrane. EVs are doubtless more complex than we imagine today, and more are still being discovered—exomeres, for example, are smaller than 35 nm, whereas apoptotic bodies are 500 nm to 2 μm. Further investigations will surely find yet more subpopulations with differing characteristics.

To ensure rigor and reproducibility in studies involving MSC EVs, 3 things must be standardized, normalized, and adhered to: first, the type of source MSC must be clearly defined; second, culture conditions must be standardized and reported; and third, EV isolation and quantification methods must be carefully considered.

Regarding EV isolation and quantification methods specifically, our laboratory has optimized a standard workflow for isolation with automated size exclusion chromatography and characterization with a combination of size distribution analysis using tunable resistive pulse sensing (TRPS) and biochemical identification using surface markers.

Many investigations focus on the use of MSC EVs in the 30- to 300-nm size range for therapeutic applications. When size exclusion chromatography is performed with automated fraction collectors, it is scalable, not dependent on density, and not only isolates that specific size range in a single go, but purity and recovery efficiency is maximized. Moreover, EVs remain intact throughout the isolation process, thus providing novel methods for normalization in any downstream application, especially, quantification and size distribution analysis and therapeutic delivery.

Positive surface markers may be confirmed by protein analyses such as western blotting or capillary electrophoresis for the following: markers clusters of differentiation (CD) 9, 63 (CD63), 68 (CD68), and 81 (CD81) are recommended and negative for the endosomal marker Golgin subfamily A member 2 (GM130) and glyceraldehyde-3-phosphate dehydrogenase.

Of the various methods that have been used to quantify EVs, TRPS is recognized as the most accurate and reliable among them. Based on the Coulter principle, TRPS measures EVs suspended in an electrolyte, particle by particle, ensuring sub-nanometer precision. Concurrently measuring both size and concentration, TRPS operates independent of measurement parameters. Moreover, TRPS quantifies absolutely, unlike other methods like nanoparticle tracking analysis or dynamic light scattering, which only provide bulk estimates. The precision of quantification is essential for downstream clinical applications and the normalization of potential dosage amounts.

CONCLUSIONS

Despite the current technical challenges facing researchers and clinicians, the results of studies of MSCs and MSC EVs will, to be sure, establish a foundation for innovative therapeutic interventions. One example of this kind of intervention is TAA. TAA is a multifactorial disease process involving many complex pathways and encompassing an array of cellular and tissue changes that fundamentally remodel a major vessel. In TAA, enhanced proteolysis causes pathologic remodeling and progressive dilation. During ECM remodeling the matrix metalloproteinas (MMPs) actively degrade the vessel wall. MMPs degrade all ECM components and activate specific growth factors and cytokines. MSCs mediate vascular ECM remodeling by suppressing multiple proteolytic enzymes, including the MMPs, and cytokines. One mechanism by which MSCs suppress proteolytic activity and cytokine release is the transfer of nucleic acids in EVs. MicroRNAs represent one of the nucleic acid subtypes transferred; they are unique in that they can target many different mRNAs and suppress translation, altering complex pathways such as cellular phenotype transformation and ECM remodeling. Figure 1 details a schematic diagram of this process.

This review—in providing a broad overview and examples of guidelines and best practices—has been a concerted...
effort to aid in overcoming technical challenges, the practical end being a collective ability on the part of the scientific and medical communities to leverage fully the protective properties of MSCs and MSC EVs to treat, ameliorate, and attenuate myriad pathologies, whereas the implicit ideological aim has been to show that stem cells are likely more capable than we imagine they might be, and that their use and implementation should only be limited by the scope of imagination and sound ethics. As such, we should endeavor always to provide the maximum benefit to human beings through scientifically rigorous investigations firmly rooted in experiential, empirical data.

Conflict of Interest Statement
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References

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