


# Innovative Diagnosis and Therapeutic Modalities: Engineered Exosomes in Autoimmune Disease

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**Abstract:** Autoimmune diseases refer to a group of conditions where the immune system produces an immune response against self-antigens, resulting in tissue damage. These diseases have profound impacts on the health of patients. In recent years, with the rapid development in the field of biomedicine, engineered exosomes have emerged as a noteworthy class of biogenic nanoparticles. By precisely manipulating the cargo and surface markers of exosomes, engineered exosomes have gained enhanced anti-inflammatory, immunomodulatory, and tissue reparative abilities, providing new prospects for the treatment of autoimmune diseases. Engineered exosomes not only facilitate the efficient delivery of bioactive molecules including nucleic acids, proteins, and cytokines, but also possess the capability to modulate immune cell functions, suppress inflammation, and restore immune homeostasis. This review mainly focuses on the applications of engineered exosomes in several typical autoimmune diseases. Additionally, this article comprehensively summarizes the current approaches for modification and engineering of exosomes and outlines their prospects in clinical applications. In conclusion, engineered exosomes, as an innovative therapeutic approach, hold promise for the management of autoimmune diseases. However, while significant progress has been made, further rigorous research is still needed to address the challenges that engineered exosomes may encounter in the therapeutic intervention process, in order to facilitate their successful translation into clinical practice and ultimately benefit a broader population of patients.

**Keywords:** engineered exosomes, autoimmune diseases, genetic engineering, anti-inflammatory applications

## Introduction

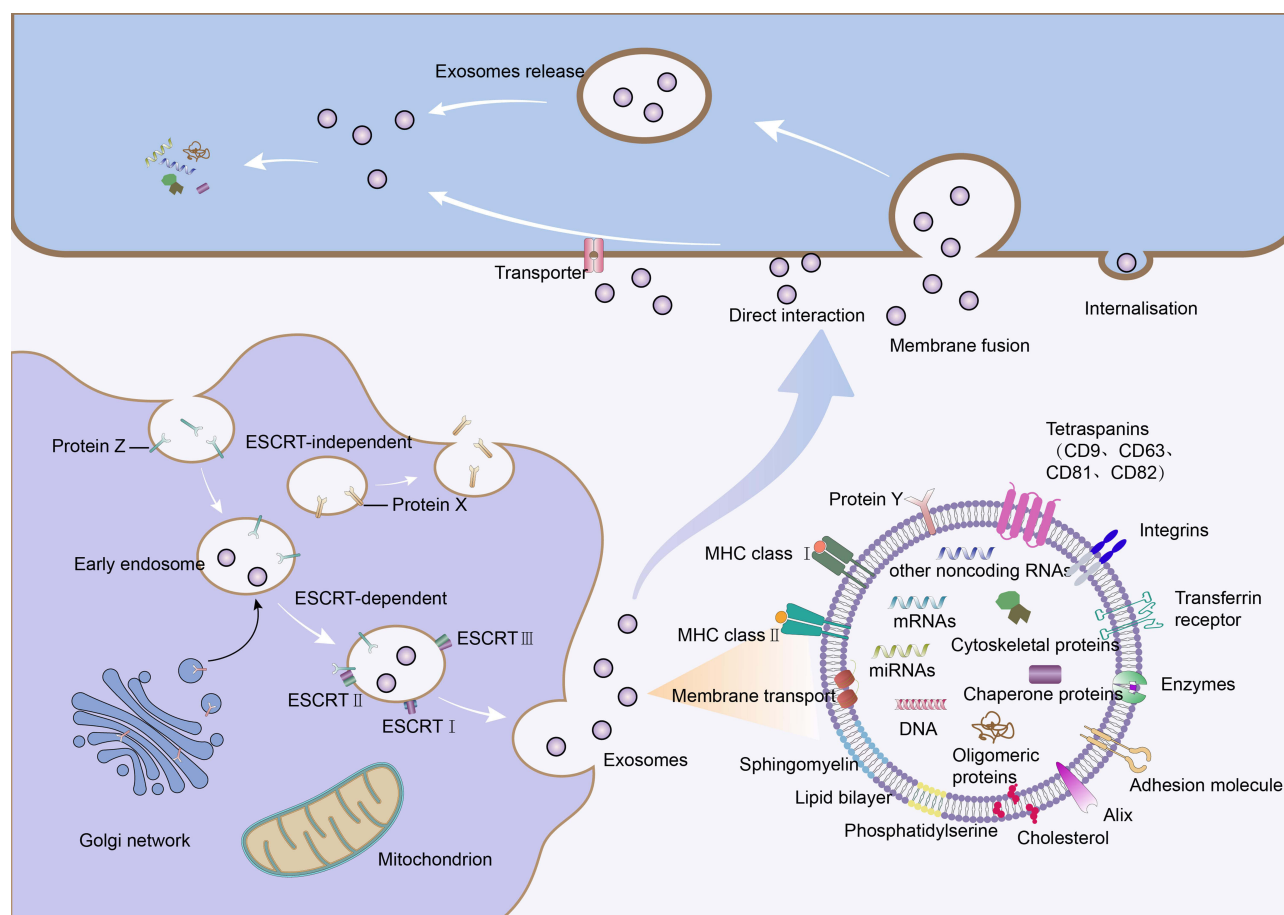
Autoimmune diseases primarily refer to a group of conditions in which the immune system's abnormal response and dysfunction lead to the attack on tissues and organs, causing damage to the body. They are one of the main contributors to the increasing mortality rates of chronic diseases. Relevant statistics indicate that the prevalence of autoimmune diseases is steadily rising, but the underlying mechanisms of these diseases remain partially unclear. This significantly hinders the prevention and diagnosis of autoimmune diseases.<sup>1</sup> Furthermore, the treatment of such diseases often involves symptomatic management and the use of immunosuppressants. However, these treatment approaches have limitations, and long-term use of immunosuppressants can increase the risk of infections and malignancies, significantly impacting the prognosis of patients. Researchers have been striving to explore new diagnostic biomarkers and treatment methods for autoimmune diseases. The emergence of exosomes and engineered exosomes offers hope in achieving this goal, particularly in the areas of treatment and drug delivery. They have the potential to partially address the shortcomings of existing treatment approaches.<sup>2</sup>

Exosomes are a type of extracellular vesicles (EVs), which are lipid bilayer membrane-bound vesicles released by cells into the extracellular space during quiescence or under stress conditions.<sup>3</sup> Exosomes are composed of a diverse

array of substances, exhibiting a wide range of biological functions. Exosomes achieve their anti-inflammatory effects through various complex mechanisms, including the secretion of anti-inflammatory proteins and cytokines, regulation of gene expression, modulation of immune cell function, and clearance of cellular debris as well as alleviation of oxidative stress. By encapsulating anti-inflammatory proteins and cytokines such as interleukin 10 (IL-10) and transforming growth factor- $\beta$  (TGF- $\beta$ ), exosomes can intervene in the activity of immune cells, inhibiting the production of inflammatory factors and thereby mitigating inflammatory responses. The release of these molecules can regulate inflammatory signaling pathways, suppress abnormal immune activation, and achieve anti-inflammatory effects. While exosomes exert excellent anti-inflammatory effects, their pro-inflammatory effects can also be seen, such as some bacteria-derived exosomes can transfer virulence factors to cause cytotoxicity, assist bacterial invasion and regulate host immune responses, which are manifested as pro-inflammatory reactions.<sup>4</sup> Additionally, exosomes carry various types of RNA, such as miRNA and lncRNA, which can regulate the expression of specific genes in target cells.<sup>5</sup> Recent studies have demonstrated that miRNAs within exosomes can inhibit the translation of inflammation-related genes by binding to the miRNAs of target cells, thereby reducing the production of inflammatory factors.<sup>6</sup> This precise gene regulation mechanism helps to maintain immune balance. Exosomes also have the ability to regulate the differentiation and function of immune cells, for example, by promoting the generation of anti-inflammatory T cells, such as regulatory T cells, and inhibiting the differentiation of inflammatory T cells, such as Th17 cells. This helps to balance immune responses and alleviate excessive inflammatory reactions.<sup>7</sup> In addition, the clearance of cellular debris and alleviation of oxidative stress are also mechanisms by which exosomes exert their anti-inflammatory effects. Exosomes help maintain cellular homeostasis and reduce inflammatory responses by engulfing cellular debris and reducing oxidative stress within cells. This contributes to the stability of the cellular environment and the attenuation of inflammation.<sup>4</sup>

Exosomes have greater potential in the field of drug delivery. Currently, drug therapy stands as one of the primary treatment modalities for the majority of diseases. Achieving optimal drug efficacy is the focal point of drug delivery research and development. Exosomes, being natural nanomaterials with inherent carrier advantages, have garnered significant attention (Figure 1) and have emerged as effective candidates for targeted drug delivery. Exosomes can evade phagocytosis and achieve prolonged circulation by utilizing their high levels of CD47, which serves as a “don’t eat me” signal. Additionally, they can trigger the CD47-SIRP $\alpha$  interaction to induce the avoidance of macrophage engulfment and clearance.<sup>8</sup> In addition, as endogenous cellular compartments, exosomes possess a wide range of cell adhesion molecules that facilitate their penetration through biological barriers. Exosomes serve as naturally occurring endogenous transport nanocarriers, offering advantages such as similarity to cell membranes, low immunogenicity, low toxicity, negative surface charge, high stability, immune evasion, and strong penetration-retention effects. In terms of biocompatibility and site-specific drug delivery, exosomes outperform synthetic nanoparticles as drug carriers. The internalization pathways of exosomes or fusion events with the recipient cell membrane may promote intracellular delivery, enabling targeted delivery of therapeutic drugs to specific intracellular compartments or organelles.<sup>9</sup> Compared to other delivery methods such as viral vectors and lipid nanoparticles, one unique advantage of exosomes is their ability to precisely deliver the payload without activating the innate or adaptive immune system. This characteristic makes repeated dosing easier and prolongs the development of patient resistance, addressing one of the major barriers encountered in recent years in gene therapy, which is drug resistance.<sup>10</sup> Furthermore, it has been discovered that mesenchymal stem cell-derived exosomes (MSC-exos) can not only inhibit the differentiation of helper T cells 17 (Th17) but also reduce the secretion of interleukin 17 (IL-17) in experimental animal models of autoimmune uveitis. This effectively prevents the progression of the disease and increases the potential of exosome-based cell-free therapies for the prevention of autoimmune diseases.<sup>11</sup>

As carriers, exosomes represent a “naturally tamed” nanocarrier, and engineered exosomes are often created through genetic engineering or chemical modification of natural exosomes. These modifications can effectively address the side effects associated with natural exosomes, optimize their biological activity, stability, and targeting ability, and further enhance their specificity and cargo delivery function. This enables them to better facilitate disease treatment and holds great potential for wide-ranging development and future prospects.<sup>12</sup> Compared to native exosomes, engineered exosomes exhibit reduced immunogenicity, minimal toxicity, and superior tissue targeting. These advantages have propelled the escalating research interest in exosomes in recent years.



**Figure 1** Parental cells (left) release exosomes, which carry bioactive molecules to the surrounding environment. Exosomes, characterized as small vesicles adorned with molecular markers, mediate the transfer of molecules such as miRNA and proteins to target cells. This interaction occurs through binding to surface receptors on the target cells or by means of endocytosis, subsequently initiating a cascade of signal transduction pathways that modulate the functions of the target cells. This process leads to immune regulation, inflammation suppression, and other biological responses.

## The Modification and Engineering of Exosomes

Currently, the engineering of exosomes primarily revolves around genetic engineering, surface modifications, and cargo loading. Genetic engineering approaches currently employed for exosomes involve gene overexpression, gene silencing, introduction of exogenous genes, and modification of signal peptides or signal sequences. These strategies allow for the editing of the cellular genome of exosomes, enabling modifications in their production, composition, and functionality. For instance, by transfecting the HEK293T cells with a plasmid containing the IL3-Lamp2b gene, exosomes overexpressing IL-3 can be generated. Subsequently, by employing siRNA targeting IL-3 receptors on cancer cells, the growth of cancer cells can be inhibited.<sup>13</sup> Exosomes can be modified by techniques such as RNA interference (RNAi) or CRISPR-Cas9 to decrease or suppress the expression of specific genes in cells, thereby altering the composition of exosomes. In the treatment of inflammatory diseases, gene silencing can be employed to reduce the levels of inflammatory factors within cells, aiding in the suppression of inflammatory responses. Research has shown that editing bone marrow-derived MSCs (BM-MSCs) using CRISPR-Cas9 to overexpress IL-10 results in BM-MSCs that effectively inhibit the infiltration of inflammatory cells and the production of pro-inflammatory cytokines in mice, thereby alleviating cellular damage.<sup>14</sup> Researchers have explored modifying the signal peptides or signal sequences within exosomes to confer them with cell-specific targeting capabilities for precise and targeted delivery of drugs or gene carriers to specific tissues or organs.<sup>15</sup>

Surface modifications of exosomes are often achieved through click chemistry or the physical interaction with lipids, allowing for surface editing of exosomes. These modifications not only streamline the purification process but also promote receptor-mediated endocytosis, facilitating specific cellular uptake and enhancing the in vivo targeting ability of exosomes. By employing metabolic engineering approaches, exosomes can be modified by covalently linking the surface azide groups with diphenylcyclooctyne-dithiophosphate. These modified exosomes can be more readily taken up by M1 macrophages and inflamed joints through the SR-A-mediated pathway, thereby reprogramming M1 macrophages into an M2 phenotype.<sup>16</sup> The physical interaction of liposomes refers to the interaction between functional groups and lipid molecules, thereby conferring functional groups onto the membrane of exosomes. For instance, the RDG peptide can interact with the dithiol phospholipid-polyethylene glycol on the surface of exosomes, allowing the introduction of biotin onto the cell membrane surface. Subsequently, drugs can be packaged into these engineered exosomes. The resulting engineered exosomes exhibit significantly enhanced selective recognition and delivery capabilities towards tumor cells.<sup>17</sup>

The cargo loading of exosomes can be achieved through passive diffusion and active encapsulation methods. Passive diffusion offers advantages such as simplicity in experimental manipulation and preservation of exosomal structure integrity. However, compared to active encapsulation, passive diffusion is limited to hydrophobic drugs and exhibits lower loading efficiency. Active encapsulation methods are commonly employed for hydrophilic compounds and large molecular substances, including techniques such as sonication, extrusion, electroporation, freeze-thaw cycles, detergent-based methods, membrane permeation, and dialysis.<sup>18,19</sup>

These engineering methods provide flexibility and controllability for the modification of exosomes to meet the requirements of different applications (Table 1). However, it is important to note that the aforementioned various engineering methods require precise technical manipulation and careful evaluation of their impact on the biological characteristics and functions of exosomes to ensure effective attainment of the desired research or therapeutic objectives.

**Table 1** The Application of Engineered Exosomes in Immunotherapy

Source	Modification Strategy	Cargo	Effects and Mechanisms
Bone marrow mesenchymal stromal cells	Surface modification	Photocrosslinked spherical gelatin methacryloyl hydrogel encapsulated cartilage affinity WYRGRL peptide <sup>20</sup>	Strengthen the inhibition of pro-inflammatory factor IL-1 $\beta$
Placental mesenchymal stem cells	Surface modification	Exosomes were encapsulated with N- (2-hydroxy) propyl-3-trimethyl chitosan ammonium chloride and oxidized konjac glucomannan polysaccharide <sup>21</sup>	Inhibition of MAPK / NF- $\kappa$ B signaling pathway enhances anti-inflammatory and tissue repair effects
Macrophages	Surface modification	Modified with both oligolysine and matrix metalloproteinase -cleavable polyethylene glycol on the membrane <sup>22</sup>	Strengthen the removal of cfDNA and promote macrophage polarization to M2 type
Chondrocyte	Membrane fusion	The chondrocyte affinity peptide was fused with the lysosomal associated membrane glycoprotein 2b protein on the surface of exosomes <sup>23</sup>	Delivery of miR-140, inhibition of cartilage-degrading proteases and alleles
Placental mesenchymal stem cells	Membrane fusion	SFBP-Gluc-MS2 was constructed by silk fibroin binding peptide, and related SGM-Exos, miR-146a-Exos and SGM-miR146a-Exos were isolated <sup>24</sup>	Effectively load miR-146a to promote chronic wound healing
Exosomes	Cargo loading	Loading OVA antigen <sup>25</sup>	Regulating CD8 + T cell immune response
Fibroblasts	Cargo loading	Loading Tripterygium wilfordii <sup>26</sup>	Reduce the expression of pro-inflammatory factors IL-6, IL-18 and IL-1 $\alpha$ Help exosomes escape the phagocytosis of MPS macrophages

(Continued)

Table 1 (Continued).

Source	Modification Strategy	Cargo	Effects and Mechanisms
B cells	Electroporation	Loading miR-155 <sup>27</sup>	The expression of TNF- $\alpha$ decreased; Effectively prevent the inflammatory induction of LPS
Adipose mesenchymal stem cells	Gene engineering	Overexpression of miR-132 by lentivirus transduction <sup>28</sup>	Inhibition of NF- $\kappa$ B p65 and I $\kappa$ B phosphorylation Promote macrophage polarization to M2 phenotype
Mesenchymal stem cells	Gene engineering	Overexpression of miR-124a <sup>29</sup>	Reduce inflammation score MH7A, RA FLSs cell lines, proliferation-related expression decreased
Umbilical vein endothelial cells	Gene engineering	Pretreatment of parental cells with TNF- $\alpha$ <sup>30</sup>	The expression level of miR-200a-3p increased through the miR-200a-3p / KLF6 / VEGFA axis.
Umbilical cord mesenchymal stem cells	Gene engineering	Overexpression of miR-146a-5p <sup>31</sup>	MiR-146a-5p acts on M1 to reduce inflammationReduce NOTCH1 expression to accelerate M2 macrophage polarization

## The Application of Engineered Exosomes in Autoimmune Diseases

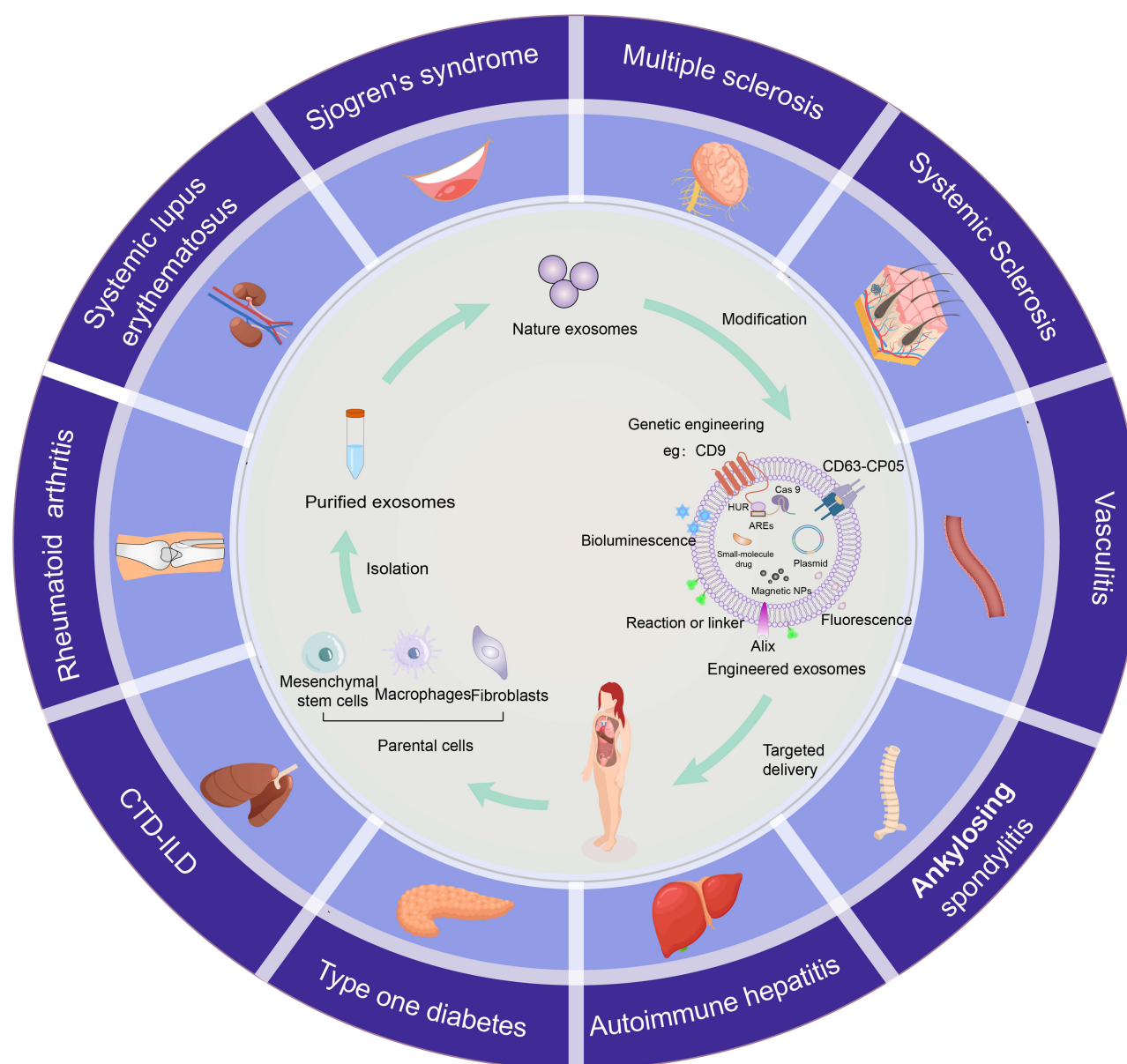
### The Applications of Engineered Exosomes in Various Autoimmune Diseases

Engineered exosomes obtained through genetic engineering and other modification methods possess enhanced functions such as inflammation suppression and tissue repair promotion, offering broad prospects for their application in various autoimmune diseases (Figure 2).

#### Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease characterized by widespread synovial hyperplasia and erosive arthritis. During the onset of RA, processes such as synovial hyperplasia, joint inflammation, and cartilage erosion contribute to disease progression. Exosomes, on the other hand, can modulate immune balance through multiple mechanisms during these processes. They can downregulate pro-inflammatory factors in serum and synovial tissues, upregulate anti-inflammatory factors to suppress synovial inflammation, and inhibit bone destruction by reducing RANKL expression while increasing the levels of osteoclast inhibitory factor.<sup>32</sup> Considering the aforementioned impact, exosomes, as a novel therapeutic approach for RA, are being progressively developed. During the progression of RA, exosomes derived from polarized M2 macrophages can mitigate inflammation by reducing the expression of pro-inflammatory factors such as interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-6 (IL-6) through various mechanisms.<sup>33</sup> The non-coding RNAs, such as let-7b-5p and miR-24-3p, can be involved in M1-M2 macrophage polarization through the Wnt signaling pathway, MAPK signaling pathway, and AMPK signaling pathway.<sup>34</sup> IL-1 $\beta$ -primed MSC-exosomes can further suppress T-cell activation and modulate macrophage polarization towards M2 by upregulating their own miR-21 and miR-146a.<sup>35</sup> The exosomes derived from adipose mesenchymal stem cells were subjected to orthogonal copper-free click chemistry for chemical modification, enabling specific binding to macrophage receptors at the site of arthritis inflammation by conjugating pyranose glucosamine and sulfate groups. Moreover, these exosomes expressed miRNAs, including let-24b-3p and miR-1-2p, facilitating the transition of macrophages from an M1 to M2 phenotype.<sup>36</sup> This process resulted in the downregulation of IFN- $\gamma$  and IL-1 expression, thereby suppressing inflammation.<sup>15</sup> IL-1 $\beta$ -primed bone marrow MSC-exosomes, through the action of miRNA-146a, are capable of eliciting similar effects, promoting the resolution of chronic inflammation.<sup>37</sup>





**Figure 2** Purified exosomes extracted from parent cells are subjected to genetic engineering and other modification methods to obtain engineered exosomes, which are then targetedly delivered to the site of action.

Engineered exosomes, in addition to enhancing their anti-inflammatory properties, have shown promising results in inhibiting bone destruction and promoting cartilage repair. An emerging therapeutic approach involves utilizing neutrophil-derived exosomes (PMN-Exo) to promote chondroprotective effects. The presence of phosphatidylserine and membrane-associated protein A1 in PMNs endows PMN-Exo with anti-inflammatory properties.<sup>25</sup> Neutrophil-derived exosomes, after undergoing nanozyme functionalization, participate in inducing the upregulation of CD4 IL-17<sup>+</sup> cells and the downregulation of CD4 Foxp3<sup>+</sup> cells. This process reduces the production of stress-induced adaptive homeostatic mediators and increases the expression of NOX-7 protein, thereby alleviating cartilage degradation caused by inflammatory arthritis.<sup>38</sup> Exosomes, with the N-terminal of the surface protein Lamp2b conjugated with a cartilage cell-targeting peptide, are engineered to form cartilage cell-targeted exosomes (CAP-Exo). Through pretreatment with sgRNA sequences, CAP-Exo can significantly inhibit the expression of MMP-13, a protease, promoting cartilage cell repair and reducing bone destruction caused by RA.<sup>39</sup>

Research on engineered exosomes in RA primarily focuses on early diagnosis and potential therapeutic applications, providing a breakthrough in alleviating the condition in RA patients. In a study, researchers administered systemically engineered exosomes derived from IL-10-modified dendritic cells (DC) to a mouse model of delayed-type hypersensitivity reaction and collagen-induced arthritis. The results revealed that the onset of collagen-induced arthritis was suppressed, and the severity of arthritis was reduced, indicating the therapeutic potential of exosomes from this source for the treatment of autoimmune diseases.<sup>40</sup> Exosomes derived from the lungs have also been found to facilitate the reconstruction of normal pulmonary alveolar structure, alleviate the fibrotic progression of interstitial lung diseases, and promote lung repair.<sup>41</sup> Evidence from relevant studies indicates significant differences in the content of exosomal components, such as *Hotair*, *MEG9*, *miRNA-17*, *miRNA-25-3p*, *miRNA-192-5p*, *miRNA-574-5p*, *miRNA-4486*, and *miRNA-451a*, between RA patients and healthy individuals. Based on these findings, early diagnosis of RA can be achieved by assessing the expression levels of these substances.<sup>42</sup> However, the detailed mechanisms underlying the role of engineered exosomes in RA still remain uncertain, necessitating further exploration by the academic community.

### Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of a large number of self-antibodies. It manifests with a diverse range of clinical presentations and can affect multiple organ systems throughout the body. The pathogenesis of SLE is associated with factors such as immune complex deposition, immune cell activation, and genetic susceptibility.<sup>43</sup> In recent years, non-coding RNAs have emerged as a hot topic in the field of rheumatology and several other disciplines, such as circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and microRNAs (miRNAs). An increasing body of evidence suggests that exosome-derived miRNAs, circRNAs, and other non-coding RNAs play significant roles in the pathogenesis of SLE.<sup>44</sup> For instance, exosome miR-146a plays a pivotal role in modulating innate immunity by targeting Toll-like receptors (TLRs), tumor necrosis factor (TNF)-related family (TNFR), and interleukin-1 receptor-associated kinase 1 (IRAK1).<sup>45</sup> Therefore, exosomes encapsulating miR-146a could serve as crucial biomarkers for SLE. Researchers have explored the use of dopamine-based surface modification on exosomes, capture molecules, and detection probes. By employing fluorescence imaging techniques, simultaneous detection of PD-1, miRNA-146a, SA, and other biomarkers carried by exosomes at the individual extracellular vesicle (EV) level has been achieved. This approach enables efficient exosome capture and simultaneous detection of multiple biomarkers.<sup>46</sup> In addition, utilizing quantitative reverse transcription-polymerase chain reaction (RT-PCR) with TaqMan probes, transferRNA (tRNA)-derived small non-coding RNA (tsRNA) have been detected in serum. Among them, tRF-His-GTG-1 exhibited a significant upregulation in the serum of patients with SLE. This finding suggests its potential application in the diagnostic evaluation of SLE.<sup>47</sup>

Glucocorticoids serve as the primary treatment for SLE, with the main therapeutic goal being the alleviation of disease progression. However, the use of glucocorticoids inevitably leads to side effects. To mitigate the harmful effects on the gastrointestinal tract, immune function, and susceptibility to pathogenic microbial infections, engineered exosomes can be employed as drug carriers. This approach offers the potential to alleviate the adverse effects associated with glucocorticoid administration to a certain extent.<sup>48</sup> Overexpressed miRNA-155 was transfected to modify MSC-exos within the CIA model. This type of exosome actively participates in suppressing autoreactive lymphocyte proliferation and enhances the expression of regulatory T cells and anti-inflammatory cytokines such as IL-10. Consequently, it further promotes the restoration of T-cell immune responses to a certain extent in SLE patients under inflammatory conditions.<sup>49</sup> The combined action of miR-4a-23p and post-transcriptional regulation of TGF- $\beta$  receptor 3 in T cells promotes the differentiation of CD2 T lymphocytes towards a regulatory phenotype. This signaling pathway inhibits T-cell proliferation and induces cell cycle arrest through the p27kip1/Cdk2 pathway.<sup>50</sup> In another study, miRNA-10a was loaded into adipose-derived mesenchymal stem cell-derived exosomes through electroporation. It was found that these engineered exosomes promoted the differentiation of naïve T cells towards Th17 and Treg phenotypes, while inhibiting their differentiation towards Th1 phenotype. MSC-exos not only possess the immunomodulatory functions of MSCs in reducing immune responses in autoimmune patients but also mitigate the potential immune rejection associated with stem cell therapy. Furthermore, they can compensate for the limitations and risks, such as instability and tumorigenicity, associated with MSC-based treatments.<sup>51</sup> However, the precise mechanisms underlying the regulatory effects of MSC-

exos remain unclear in current research. The engraftment survival rate and safety profile of MSC-exos have not yet reached the desired level, necessitating further exploration by researchers.

### Sjögren's syndrome

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by progressive destruction of exocrine glands, with the main clinical manifestations being dry mouth and dry eyes. It involves multiple organs.<sup>52</sup> The current primary diagnostic criteria for this condition rely on characteristic clinical symptoms and relevant antibody testing. Non-invasive collection of exosomes from patient tears allows for early diagnosis and prediction of ocular diseases through the utilization of liquid biopsies and nanotechnology-based analysis.<sup>53</sup> The introduction of the engineering technique called nanoporous membrane-based resonators, or iTEARS, enables rapid and high-yield purification of exosomes from small volumes of tear fluid. This technology also allows the identification of various components such as miR-145-5p, miR-214-3p, miR-218-5p, and miR-9-5p as potential biomarkers for ocular diseases. Furthermore, these exosomal samples can be used for regular analysis to monitor disease progression and treatment efficacy.<sup>54</sup>

The treatment process for patients with SS is lengthy, and current therapies often rely on immunosuppressants, artificial saliva eye drops, and nonsteroidal anti-inflammatory drugs, yet an ideal treatment approach has yet to be established. MSCs and exosomes have emerged as a research focus for SS treatment, owing to their remarkable immunomodulatory capabilities. MSC-derived exosomes possess similar immune-regulatory and tissue repair functions as MSCs. For instance, MSC-exos target the NF- $\kappa$ B pathway to enhance Treg cell and Th2 cell responses, holding potential for promoting functional restoration of salivary glands in SS patients.<sup>55</sup> In addition, MSC-exos enhance the inhibitory function of bone marrow-derived suppressor cells by upregulating arginase expression and releasing S100A4 bound to TLR4, thereby promoting the secretion of IL-6. This mechanism significantly delays the progression of the disease in SS patients.<sup>56</sup> Exosome therapy offers numerous advantages over cellular-based approaches, providing multiple avenues for the treatment of SS. However, the in-depth application of engineered exosomes in this disease is still under investigation and requires further research.

### Systemic sclerosis

Systemic sclerosis (SSc) can be classified into two subtypes: diffuse systemic sclerosis and limited systemic sclerosis. Fibrosis of the skin or internal organs serves as the predominant pathological feature of SSc.<sup>57</sup> miRNAs play a crucial regulatory role in various biological processes by post-transcriptionally interfering with gene expression. Therefore, they represent potential novel therapeutic targets for disease progression mitigation. Kevin et al employed genetic engineering to overexpress miRNA-let7c in MSC-exos and selectively delivered them to damaged renal cells. This strategy effectively inhibited the actions of TGF- $\beta$ 1 and the expression of  $\alpha$ -SMA gene, while simultaneously inducing a reduction in fibrotic gene expression levels. As a result, it effectively alleviated renal damage and fibrotic progression.<sup>58</sup> The members of the miR-let7 family, including miR-let7b and miR-let7c, also play a similar role in diabetic nephropathy-induced renal fibrosis through the TGF- $\beta$ R1 and TGF- $\beta$ /Smad signaling pathways.<sup>59</sup> A study utilized a mouse model of liver fibrosis induced by hepatic stellate cells. Through genetic engineering, adipose-derived MSCs with high expression of miRNA-181-5p were engineered. The transfer of miRNA-181-5p through exosomes resulted in the inhibition of TGF- $\beta$ 1-induced NRK52E cells. This intervention partially alleviated fibrosis in the mouse model, indicating the potential therapeutic value of exosomes in the treatment of fibrosis.<sup>60</sup> The progression of SSc involves multiple tissue organs and is closely associated with the tissue microenvironment. The tissue-specific and non-immunogenic delivery of engineered exosomes holds great potential for advancements in the treatment of SSc, though further exploration of its applications is required.

### Vasculitis

Vasculitis refers to a group of inflammatory autoimmune diseases characterized by inflammation of the blood vessel walls. Autoantibodies play a crucial role in the pathogenesis of vasculitis, with anti-neutrophil cytoplasmic antibodies (ANCA) being the most significant among them. Extracellular vesicles (EVs) are extensively involved in various regulatory pathways, and their association with the dissemination and maintenance of vasculitis-related inflammatory



reactions is well established. Research by Kahn et al revealed that EVs derived from various cell sources can transport functional B1 receptors to target cells, thereby promoting the occurrence and propagation of inflammation.<sup>61</sup> Exosomes can also influence innate immune cells and adaptive immune cells by transporting various immune molecules, thereby disrupting the immune homeostasis within the body. Loading PR3 and myeloperoxidase (MPO) into EVs can serve as antigenic stimuli, eliciting an immune response and participating in the immune reactions associated with vasculitis.<sup>62</sup> Gerjan et al have also observed that patients with vasculitis carry exosomes overexpressing miRNA-142-3p, which leads to impaired functionality of Treg cells.<sup>63</sup> Based on the aforementioned findings, the relevant exosomes have the potential to become therapeutic targets for vasculitis. Antoniadis and Grimsey conducted a study and found that constructing exosome-packaged p38 inhibitory peptides can suppress p38 signaling, thereby blocking a series of inflammation induced by G-protein coupled receptor activation in vasculitis.<sup>64</sup> As a drug delivery system, nano-vesicles loaded with TPCA-1 (NF- $\kappa$ B inhibitor) can significantly alleviate symptoms in a mouse model of vasculitis.<sup>65</sup> By employing donor cell-assisted membrane engineering strategies, engineered exosomes obtained with membrane modifications such as the Arg-Gly-Asp (RGD) peptide exhibit enhanced targeting towards blood vessels. These engineered exosomes not only possess the ability to promote vascular repair and regeneration but also demonstrate increased specificity towards the vasculature due to the aforementioned RGD peptide modification on their exosomal membrane.<sup>66</sup> The prospects for engineered exosomes as a next-generation therapeutic target and drug delivery vehicle for vasculitis are extremely promising.

### Ankylosing spondylitis

The pathological mechanisms of ankylosing spondylitis (AS) primarily involve pathological ossification and an inflammatory environment, particularly affecting the sacroiliac joints. Liu et al engineered M2 macrophages using miRNA-22-3p and utilized exosomes to transport them to AS mesenchymal stem cells (MSCs). They discovered that miRNA-22-3p, by downregulating the expression of PER2, further stimulates MSCs to undergo osteogenesis through the Wnt/ $\beta$ -catenin axis, promoting the occurrence and development of ankylosing spondylitis.<sup>67</sup> In the early stages of AS, the cartilage of the sacroiliac joints undergoes erosive destruction, which is considered an initial manifestation of ankylosing spondylitis. Research has shown that the fusion of MSCs with peptide E7 and exosomal membrane protein Lamp 2b yields engineered exosomes with targeting capabilities similar to synovial fluid-derived mesenchymal stem cells (SF-MSCs). These engineered exosomes enhance the effective concentration and uniform distribution of Kartogenin within cells, thereby further accelerating SF-MSCs' cartilage formation process.<sup>68</sup> This approach also holds promise for advancing the treatment of AS.

### Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM), also known as insulin-dependent diabetes, is an autoimmune disease characterized by elevated blood glucose levels as the primary clinical manifestation. Prior to the onset of noticeable clinical symptoms in T1DM patients, latent autoimmune responses have already begun within the body. Therefore, it is highly significant to utilize T1DM biomarkers for prevention and treatment during the asymptomatic phase. Alexander et al demonstrated that miRNA-21-5p gradually increases in the early inflammatory environment of T1DM, showing tremendous potential as a biomarker for T1DM.<sup>69</sup> Scholars have utilized electroporation to load miRNA-21-5p mimics into exosomes derived from human adipose-derived stem cells. These engineered exosomes exert their effects on the Wnt/ $\beta$ -catenin signaling pathway, promoting proliferation and migration of keratinocyte cells, facilitating collagen remodeling and angiogenesis, thus further accelerating wound healing in patients with T1DM.<sup>70</sup>

Furthermore, considering the pathogenesis of T1DM, its treatment approach should focus on rebuilding immune tolerance, suppressing lymphocyte clearance or damage to pancreatic beta cells, and increasing insulin levels in the body. Regeneration of pancreatic beta cells is limited in patients with T1DM. Sohei et al conducted in vivo experiments and found that adipose tissue-derived MSCs-EXOs have the capability to increase the quantity of regulatory T cells and their products, thereby providing protection for T1DM mice.<sup>71</sup> Moreover, recent animal experiments have shown that exosomes have beneficial effects on improving diabetes complications. Exosomes derived from rat bone marrow mesenchymal stem cells have the potential to repair damaged neurons and astrocytes, improving cognitive impairment in diabetic mice and reversing their functional deficits.<sup>72</sup> In a study, miRNA-engineered umbilical cord-derived MSC-exos were prepared using electroporation and injected into a rat model of diabetic retinopathy. These exosomes carried

miRNA-102, which regulated PGC-1 $\alpha$ .<sup>73</sup> In comparison to the control group, these exosomes demonstrated enhanced anti-apoptotic and anti-DNA damage properties, as well as protective effects on the retinal barrier. They effectively slowed down the progression of diabetic retinopathy.<sup>69,70</sup> Even more encouragingly, exosomes have the potential to improve the success rate of pancreatic islet transplantation. Di et al discovered that exosomes derived from human bone marrow mesenchymal stem cells (hBMSCs) transfected with anti-miRNA-375 can enhance the function of regulatory T cells and inhibit the proliferation of peripheral blood mononuclear cells (PBMCs), which is beneficial for promoting pancreatic islet transplantation.<sup>70</sup> However, the different cell origins of exosomes can also influence their distribution. For example, administration of exosomes through the peritoneal cavity or muscle can reduce the accumulation of exosomes in the liver, but it may also increase the content of exosomes in target organs such as the pancreas.<sup>72</sup>

## Engineering Exosomes Combined with Other Materials are Used in Autoimmune Diseases

Exosomes, as engineered extracellular vesicles, have emerged as a new generation Drug Delivery System (DDS) due to their low immunogenicity, high delivery efficiency, and specific targeting advantages. These engineered exosomes possess certain characteristics and essential components from their parent cells, enabling them to exert their therapeutic effects on target cells through various mechanisms. This innovative approach has facilitated novel treatments for numerous diseases.<sup>73</sup> For instance, Hybrid Exosomes (HEs) can enhance intercellular interactions by altering the lipid composition or utilizing specific properties of exogenous lipids. The resulting nanocarriers can be applied in advanced Drug Delivery Systems (DDS) for various purposes.<sup>74</sup> Compared to previous research findings, Piffoux et al have achieved significant advancements in their study. They successfully engineered hybrid carriers by chemically attaching liposomes to exosomes and combining them with polyethylene glycol (PEG). This hybrid vehicle not only effectively enriches the EVs but also retains their original properties. Furthermore, these hybrid carriers have demonstrated superior performance in DDS.<sup>75</sup> Combining magnetic nanoparticles made of superparamagnetic or ferromagnetic Fe<sub>3</sub>O<sub>4</sub> or  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> with drug-loaded exosomes offers the possibility of targeted drug delivery with reduced toxic side effects and localized drug release. These magnetic nanoparticles possess small size, high sensitivity, low toxicity, stable performance, and readily available source materials. In recent years, the potential for targeted drug administration using these magnetic nanoparticles has been steadily increasing. This approach not only allows for precise drug release at specific sites but also reduces the overall dosage of the drug.<sup>76</sup> In addition, the dense glycocalyx coating on the outer layer of exosomes holds promising potential for enhanced targeting through engineered modifications. Sialyl Lewis-X (sLeX), which is involved in leukocyte adhesion to endothelial cells expressing E-selectin during inflammation, has shown promise as a targeting ligand. Through genetic engineering of exosomes-producing cells, endogenous sorting of the targeting ligand to the exosomes can occur, thereby facilitating surface expression of sLeX and characterizing their ability to activate endothelial cells for targeted delivery.<sup>77</sup>

With the advancement of exosome research, many scholars have discovered that exosomes, in addition to their characteristic transport functions, also have an enhancing effect on the therapeutic properties of certain materials. Hydrogels, due to their excellent barrier function and selective semi-permeability, can serve as ideal dressings for chronic wounds in autoimmune diseases. Meanwhile, microporous annealed particle (MAP) scaffolds, as a type of flowable and in-situ crosslinked porous scaffold, can be combined with hydrogel microbeads composed of L- or D-chiral peptides. This combination induces T cell-dependent IgG1 and IgG2a reactions, enhancing both innate and adaptive immune responses and promoting skin regeneration.<sup>78</sup> The combination of chondroitin sulfate (CS) or regenerated silk fibroin (RSF) with hydrogel exhibits enhanced adhesive properties. When these composites are loaded with BMSC-exos and released at the site of cartilage defects, they can stimulate the proliferation of BMSCs and their differentiation into chondrocytes.<sup>79</sup> Polydopamine, due to its high adhesiveness and excellent biocompatibility, is often chosen as a coating for scaffolds in combination with polylactic-co-glycolic acid (PLGA). The tissue engineering system composed of exosomes and this scaffold is beneficial for bone regeneration in mice.<sup>80</sup> This combined application opens up a new pathway for gene therapy against osteoporosis, offering improved biocompatibility and demonstrating high cellular targeting specificity and efficient transmembrane delivery. Plant exosomes containing curcumin have been employed to

investigate the ability of plant exosomes to deliver curcumin to normal and colorectal cancer tissues. Additionally, exosomes derived from antigen-loaded dendritic cells can be used in experiments involving vaccine administration using dendritic cell-derived exosomes loaded with tumor antigens.<sup>81</sup>

In addition to the aforementioned extracellular materials, the cellular components themselves can also interact synergistically with exosomes. Extracellular matrix (ECM), exosomes, and growth factors are all important factors that influence tissue repair and regeneration in the human body. The ECM intervenes in the body's repair process by regulating the phenotype and expression of stem cells.<sup>82</sup> Exosomes, on the other hand, exert their influence on this process through the utilization of miRNAs and proteins. The ECM, with its characteristic physiological structure, can serve as a scaffold for exosomes, facilitating the loading of exosomes to maintain their activity. This helps to overcome the limitations of exosomes, such as their high diffusibility and short half-life. By utilizing the ECM as a supportive matrix for exosomes, it enhances the repair and regeneration of human tissues.<sup>83</sup>

## Conclusion

Exosomes, as a potential biomarker and therapeutic target for autoimmune diseases, have attracted extensive research attention from the medical community. Current studies on engineered exosomes largely focus on harnessing the low immunogenicity of natural exosomes and specifically modifying certain miRNAs to enhance their transport and reduce degradation. This promotes the post-transcriptional expression of miRNAs and regulates the expression of corresponding target proteins. By modulating various target proteins, multiple signaling pathways can be regulated, thus further alleviating the progression of autoimmune diseases. Additionally, efforts are being made to explore how to fully utilize the cargo capacity of engineered exosomes to effectively enhance the therapeutic efficacy of existing drugs.

Exosomes derived from different cell types, after various modifications and processing, exhibit more effective, stable, and safe immunomodulatory effects in the progression of autoimmune diseases. However, research on engineered exosomes is still in its early stages, and some findings have not yet reached definitive conclusions. There are many challenges to fully applying them in the diagnosis and treatment of autoimmune diseases. In addition to issues related to isolation, production, storage, and preservation of engineered exosomes, there are several challenges in their clinical application for autoimmune diseases. These include the storage and maintenance of engineered exosome activity, the effect of disease conditions on the metabolic rate of engineered exosomes in the human body, the safety and specificity of therapeutic methods related to engineered exosomes in clinical settings, all of which require resolution. In the future, researchers should continue to explore the potential value of engineered exosomes in autoimmune disease-related treatments, such as their transport targeting, circulation stability, and biocompatibility. This will promote the development of clinical applications for engineered exosomes, allowing them to fully demonstrate their value in autoimmune diseases.

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## Disclosure

The authors report no conflicts of interest in this work.

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