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Engineered Exosomes for Targeted Protein Delivery in Rheumatoid Arthritis

Kaitong Xie

Shenzhen Middle School, Shenzhen, China
ketyxie@gmail.com

Abstract. Rheumatoid arthritis is a sysrenmic autoimmune disease which mainly affects joints that affects millions of patients in the world. Protein therapeutic is one of the important treatment against this disease. In order to perform precised protien delivery, engineered exosomes are significant tools. This review includes the pathophysiology of rheumatoid arthritis and therapeutic targets, introduction of exosomes, strategies of engineering exosomes and the evaluation of engineered exosome-based protein drug delivery systems. The research shows that exosome is a good material for target protein delivery due to its accuracy and biocompatibility. The efficiency is significantly increased comparing with other delivery system. However, there are still some limitation for this method, such as high cost and low cargo loading efficiency. This review calls for the medical industry's attention on this kind of new technique to improve the engineeringf strategies and reduce the costs, in order to put this method in use for more patients.

Keywords: Rheumatoid arthritis, exosome, extracellular vesicle, biomedical engineering

1. Introduction

Rheumatoid arthritis (RA) represents a systemic autoimmune disorder. The main symptoms are persistent joint inflammation, progressive cartilage destruction and irreversible functional disability. The disease will influence other systems including pulmonary, ocular and cardiac complications as it develops. According to global epidemiological statistics, about 17.6 million individuals worldwide lived with RA in 2020, and patient prevalence is projected to reach 31.7 million by 2050 [1]. Current clinical includes small-molecule agents and biological protein therapeutics, yet considerable practical bottlenecks remain unaddressed. Conventional protein drugs, for example, anti-cytokine biologics, have limitations such as rapid systemic clearance, off-target immune suppression and insufficient accumulation within inflamed joint tissue, which compromise overall therapeutic outcomes [2].

Researches all over the world have explored many kinds of nanocarrier platforms to overcome protein delivery limitations for RA treatment. Liposomes, polymeric nanoparticles and lipid nanoparticles exhibit favourable loading capacity, but they are limited by low biocompatibility, high immunogenic risk and limited tissue penetration into inflamed synovium [3]. These days, the advantages of exosomes have highlighted there roles as promising endogenous delivery carriers. As they are screted by cells, exosomes possess high biocompatibility, low immunogenicity and the

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capability to shield encapsulated biomolecules from enzymatic degradation [4]. Multiple overseas investigations have shown that surface-modified exosomes achieve joint-targeted accumulation in collagen-induced arthritis mouse models, and exosome-loaded anti-inflammatory proteins generate superior local anti-arthritic effects compared with free protein administration[5, 6]. Domestic research groups have further optimised pre-loading and post-loading engineering workflows, demonstrating mesenchymal stem-cell-originated exosomes as preferable candidates for RA-oriented protein cargo encapsulation, whereas critical gaps persist regarding loading efficiency, large-scale manufacturing standardisation and in-vivo protein-activity retention [7].

Existing reviews mostly summarise general exosome-based RA therapy, while systematic summaries focusing specifically on engineered exosomes for therapeutic-protein targeted delivery remain scarce [8]. In this review, the aim is to summarize the efficiency of exosomes in precised protein delivery, outline its advantages than other methods, present the strategy of exosome protein deliveries and point out the future challenges of this technique.

2. Pathophysiology of rheumatoid arthritis and therapeutic targets

Rheumatoid arthritis, a chronic systemic autoimmune disease. In the beginning of the disease, it mainly affects the lining of the synovial joints. As the disease develops, the possibility of disability also progresse. Premature death can also happen. And there will be heavy socioeconomic burdens. Self-reactivity is an important cause of RA. There are numerous studies prove that chronicity of RA disease is caused by dysfunctions not only in the adaptive and innate immune systems, but also the nalterations in stromal cells [2]. In early stages, although there might be no clear symptoms, some genetic factors can contributed to the susceptibility to RA [2]. There's no clear proof of the environmental factors will be a certain cause of RA, but high risk factors such as smoking will increase the risk of this disease. Post- translated modifications such as deamination and citrullination of proteins involve in the development of RA [2]. No definitive causal relationship has been confirmed for environmental triggers, yet risk factors such as smoking raise disease incidence. Post-translational modifications including protein deamination and citrullination participate in RA pathological progression [2]. Altered peptides are presented as autoantigens, driving T-cell activation and subsequent B-cell stimulation, which promotes the generation of anti-citrullinated-protein autoantibodies [2]. These circulating autoantibodies including rheumatoid factors (RF) can persist for up to 10 years before clinical symptom onset [2].

In early RA, immune cells secret cytokines and these molecules causes the inflammation of the patients' joints. Mononuclear cells infiltrate the synovial membrane of the joints [2]. Over time, it develops into the classic form of RA. There are two key modifications that play important roles in the pathogenesis of the disease. The first modification is the proliferation of synovial membrane cells. This causes an increase of the number of activated macrophage-like synoviocytes. They release inflammatory cytokines such as interleukin (IL)-6, IL-1, and tumor necrosis factor alpha (TNF-alpha) [2]. The other modification is the infiltration of the lymphocytes. They enters the lining of the synovial membrane. Nearly 50% of these cells will finally form memory CD4+T cells and B-cells are circulated in these centers [2]. Those B-cells turns into plasma cells through differentiation and different types of anti bodies are produced, eventually causing cartilage damage, which is the main symptom of RA [2].

Multiple protein-based therapeutics have been developed for RA intervention. For instance, Anakinra targets pro-inflammatory IL-1; neutralisation of this cytokine relieves local joint inflammation. Another biological agent Adalimumab acts against tumor necrosis factor alpha. By binding to both soluble and membrane-bound TNF-α, Adalimumab neutralises inflammatory

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signalling and mitigates joint tissue damage. Nevertheless, non-targeted systemic distribution of therapeutic proteins disrupts physiological immune homeostasis because cytokines undertake essential immune-regulatory functions. Insufficient targeting capability also lowers effective drug concentration at lesion sites and reduces overall therapeutic potency [4]. Accordingly, novel delivery approaches capable of precisely transporting therapeutic proteins to inflamed joint tissue are urgently required.

3. Exosomes as natural drug delivery vehicles

Exosomes are nanoscale extracellular vesicles secreted by cells [3]. They are enclosed by phospholipid bi-layers and contains various biologically active cargoes (protein, lipid nuclear acids, etc.) [3] As exosomes are secreted by cells, they have high biocompatibility, low immunogenicity and low toxicity. The phospholipid membranes allow them to move across biological barriers. They can also weakly react to the carried proteins to prolong the proteins' cargo half-life [4]. By modifying gene or chemical engineering the surface of the exosomes, the ability of targeted drug delivery could be enhanced [4]. Adding signal molecules to the surface of the exosomes allows them to enter specific cells and release the medical protein that contains within it, accomplish accurate protein delivery. Genetic modified parent cells can produce the exosomes needed. Those exosomes show promise as drug delivery vehicles, and have been used to treat neurological, cardiovascular, cancer, and immune diseases [3].

4. Engineering strategies for exosome-based drug delivery systems

4.1. Cargo loading strategies

Two principal cargo-loading approaches are available for exosome engineering: pre-loading and post-loading strategies. For pre-loading workflows, parent donor cells are genetically engineered to produce cargo-containing exosomes [5]. These approaches support continuous production of cargo-loaded exosomes without compromising membrane integrity [5]. Pre-loading can be achieved either through cell-cargo co-incubation or genetic modification of donor cells [5]. Co-incubation protocols are more applicable for hydrophobic small-molecule compounds [5]. Therapeutic agents are co-cultured with parent cells and traverse biological membranes to reach multivesicular bodies (MVBs), the intracellular compartment where exosomes are generated [5]. Cargo-loaded exosomes are subsequently isolated via differential centrifugation for downstream applications. This method features simple operation and low experimental cost [5]. However, it suffers from low loading efficiency and relies heavily on transmembrane concentration gradients of loaded substances [5]. Genetic-modification-based pre-loading requires transfection of engineered plasmids into donor cells. Therapeutic moieties including RNAs, proteins, ligands and receptors are overexpressed inside parent cells via genetic transfection [5]. Tumour-derived cell lines and stem-cell populations are commonly utilised for this technical route [5].

Regarding post-loading strategies, cargo substances are loaded into already-isolated exosomes via active or passive loading modalities [5]. Native exosome particles are harvested by centrifugation procedures. Active loading requires cargo molecules to cross exosomal lipid bilayers [5]. Active-loading techniques are divided into physical-induction and chemical-induction categories [5]. Physical-induction approaches temporarily disrupt exosomal membranes by external mechanical force [5]. Chemical-induction-based protocols achieve relatively high loading efficiency yet carry potential cytotoxic risks. Passive loading relies on co-incubation between purified exosomes and

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highly-concentrated cargo molecules [5]. Cargo candidates for passive loading are generally hydrophobic substances capable of spontaneous diffusion across lipid bilayers [5]. This protocol remains cost-effective and technically accessible, yet it still bears intrinsic limitations including unsatisfactory loading efficiency.

4.2. Surface engineering for targeting

Membrane modification is really essential if you want to achieve precise targeted delivery inside the body [6]. Over the years, quite a few techniques have been developed to modify exosome membranes, including genetic engineering, lipid insertion, click chemistry, metabolic labelling, affinity-based binding and enzymatic ligation [6]. But in this section, it mainly going to focus on genetic-modification-based strategies. To do this, you first need genetically engineered donor cells. Basically, you genetically fuse target-binding peptides with exosomal-membrane proteins or lipid-binding protein domains [6]. As a result, the exosomes secreted by these modified cells end up displaying targeting moieties on their surface [6].

These genetic-engineering-based strategies can be roughly divided into two categories: membrane-protein-dependent approaches and lipid-protein-interaction-dependent ones [6]. Some proteins are commonly found on exosomal membranes, like CD63, CD81, CD9 and Lamp2b [6]. Among these, Lamp2b is one of the most popular choices for displaying targeting peptides [6].

It's also possible to modify the lipid components of the exosomal membrane. In this case, targeting peptides are genetically fused with lipid-binding proteins [6]. After that, the modified donor cells produce exosomes that carry the targeting moieties through peptide-lipid interactions [6]. However, this approach isn't as straightforward and is used less often compared to the membrane-protein-based method [6].

4.3. Improving stability and circulation time

The practical delivery performance of exosomes is constrained by their biological stability and circulating half-life. Multiple modification approaches address these limitations, including albumin-binding-peptide display, PEGylation, CD47 overexpression, lipid-anchor DOPE modification, microgel encapsulation and lyophilisation. This section concentrates on the albumin-binding-peptide-display strategy.

Following systemic administration, unmodified native exosomes predominantly accumulate within hepatic and splenic tissues and possess relatively short circulating half-lives. Extended systemic circulation represents a prerequisite for sufficient exosome enrichment within inflamed joint lesions in RA animal models [7]. Human albumin exhibits an approximately three-week circulating half-life in vivo [7]. Genetically encoded albumin-binding-peptide fusion constructs are assembled into lentiviral vectors for stable donor-cell transfection. Engineered HEK-293T cell lines continuously produce exosomes displaying albumin-binding domains on their outer surface [7]. Compared with native exosomes, these engineered vesicles achieve greatly prolonged circulation duration. Plasma retention of ABD-CD63-modified exosomes reaches 10-fold higher levels relative to native exosomes 270 minutes after in-vivo injection [7].

4.4. Cell source selection for engineered exosomes

Mesenchymal stem cell-derived (MSC-derived) exosomes are chosen. This kind of exosomes have the function of protocells and lower immunogenicity. They have a wide range of therapeutic

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advantages above other cell therapies [8]. Recently, they have been the most studied cell type for therapeutic needs, and have played a important role in treating many kinds of diseases including, kidney injury, graft-versus-host disease (GVHD) immune tolerance, nerve injury, tissue and organ transplantation, rheumatic disease, and liver disease [8]. Despite from low immunogenicity, MSC-derived exosomes also have a lot of other advantages, such as long-circulating half-life, small size, remarkable penetration, and biocompatibility.

5. Evaluation of engineered exosome-based protein drug delivery systems

5.1. In vitro characterization

Exosome samples are characterised with respect to particle dimension, morphological features, protein cargo composition and canonical biomarker expression via dynamic light scattering, nanoparticle tracking analysis, cryogenic transmission electron microscopy and Western blotting according to standard experimental workflows [3].

5.2. In vivo efficacy evaluation

Choi et al. evaluated the therapeutic potential of exosomes derived from human adipose tissue-derived mesenchymal stem cells (iMSCs) that were primed with serum from a non-human primate rheumatoid arthritis (RA) model [9]. They administered these exosomes (termed Exo-RA) to CIA model mice and compared their effects against exosomes from non-primed iMSCs (Exo-FBS) and methotrexate [9]. Notably, Exo-RA exhibited significantly higher TGF-β1 content and a greater number of exosomes than Exo-FBS [10]. In vivo, Exo-RA treatment led to a significant increase in serum IL-4 levels and the proportions of Th2 and M2 cells, alongside a marked reduction in pro-inflammatory cytokines, including TNF-α, KC, and IL-12p70 [9]. These findings collectively suggest that Exo-RA effectively alleviates cartilage damage in the CIA model [9].

Chen et al. Evaluated the IFN-γ-primed MSC extracellular vesicles attenuate rheumatoid arthritis via PD-L1-driven T-cell suppression and bone preservation. In their research, data illustrates that IFN-γ-EVs can significantly reduce the bone erosion, joint inflammation and proinflammatory cytokine levels in CIA mice, which is more efficient than native MSC-EVs [10]. IFN-γ priming upregulates programmed death-ligand 1 (PD-L1) on the EVs [10]. Furthermore, the subsequent genetic ablation of PD-L1 completely abolished these therapeutic benefits [10].

5.3. Safety and biocompatibility

The safety and therapeutic potential of mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) in rheumatoid arthritis (RA) have been preliminarily verified in multiple early-phase clinical trials [11]. A growing body of clinical evidence supports the favorable safety profile of MSC-EVs. As reviewed by Yang et al. (2026), available clinical data demonstrate satisfactory tolerability toward MSC-EVs among RA participants, with no severe adverse events reported throughout the trials [11]. Nevertheless, current clinical studies exhibit notable restrictions such as limited sample scale and the absence of unified administration-protocol standards [11].

Li et al. reported that M2-exosome-based nanocarriers loaded with BSP-encoding plasmid DNA exhibit prominent accumulation within inflamed joint sites in RA mouse models, exerting strong anti-inflammatory bioactivity and desirable therapeutic potency [12]. It should be noted that this platform delivers nucleic-acid cargo rather than therapeutic protein molecules. Toxicological

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assessments indicate no detectable cytotoxicity both in vitro and in vivo for this vesicle-based delivery system [12]. Collectively, appropriately engineered exosomes display promising safety and biocompatibility profiles, qualifying them as prospective delivery candidates for RA-targeted therapeutics.

6. Challenges and future perspectives

Despite the promising potential of engineered exosomes for RA therapy, there are still a number of practical issues that need to be sorted out used in the clinic. One major issue is that the production process hasnot really been standardised yet. Right now, it's expensive and the yield is pretty low, which is why this approach still is not widely adopted. Another bottleneck is the relatively low efficiency when it comes to loading therapeutic cargo into the exosomes. There's also the question of safety—things like off-target effects, potential immunogenicity, and product heterogeneity still need to be carefully looked at.

To move this field forward, standardisation really should be the top priority. That means setting up consistent, end-to-end protocols for every step—starting from choosing the right cell source all the way through to the final formulation. At the same time, it need to dig deeper into how exosomes actually interact with the immune microenvironment in RA, which would help guide more rational design choices. It is also worth exploring alternative production platforms, like plant-derived exosomes, which could bring down costs and offer scalability with different cargo profiles. And of course, there is still a lot of room for improvement when it comes to personalisation and precision in dosing.

7. Conclusion

This paper underscores the considerable promise of engineered exosomes as targeted delivery platforms for therapeutic proteins in the context of rheumatoid arthritis. The pathophysiology of RA is characterised by immune dysregulation, chronic synovial inflammation, and the overproduction of key pro-inflammatory cytokines—including TNF-α, IL-1, and IL-6—which collectively contribute to progressive joint degradation. Although currently available protein therapeutics such as Anakinra and Adalimumab are capable of neutralising these inflammatory mediators, their clinical utility is constrained by suboptimal delivery efficiency and frequent off-target effects.

Exosomes, as naturally occurring nanoscale extracellular vesicles, offer several distinct advantages over conventional synthetic delivery systems. Their low immunogenicity, excellent biocompatibility, and ability to traverse biological barriers render them particularly attractive for targeted drug delivery. Over recent years, a range of engineering strategies have been developed to further enhance their therapeutic performance. These include pre-loading and post-loading techniques for cargo encapsulation, genetic and chemical surface modifications to improve targeting specificity, albumin-binding peptide display to prolong systemic circulation, and the adoption of mesenchymal stem cells as a scalable and reliable cell source.

Preclinical evidence has demonstrated that engineered exosomes can selectively accumulate in inflamed joints in collagen-induced arthritis models and effectively attenuate cartilage damage through immunomodulatory mechanisms and cytokine neutralisation. Moreover, early-phase clinical trials have provided preliminary evidence supporting the safety and tolerability of MSC-derived exosomes, with no severe adverse events reported to date.

Nevertheless, several critical obstacles must be addressed before clinical translation can be realised. These include low drug loading efficiency, elevated production costs, batch-to-batch

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variability, and the absence of standardised manufacturing protocols, all of which currently impede the widespread adoption of this approach.

Future research efforts should prioritise the development of scalable and reproducible production systems, the exploration of alternative sources such as plant-derived exosomes to reduce manufacturing costs, and the advancement of personalised exosome formulations tailored to individual patient needs. With continued scientific innovation and interdisciplinary collaboration, engineered exosomes hold substantial potential to emerge as a next-generation therapeutic modality, ultimately improving both treatment outcomes and quality of life for RA patients worldwide.

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