"Emerging Vesicular Nanocarriers for Tofacitinib: A Novel Approach to Enhancing Therapeutic Outcomes in Rheumatoid Arthritis"

Shivani Pannu[1,2], Pooja[2], Reena[2], Rahul Sharma[2], Inderjeet Verma[1]*

1. MM College of Pharmacy, MMDU, Mullana, Ambala, Haryana
2. Gateway College of Pharmacy, Sonipat, Haryana

*Corresponding Author- Dr. **Inderjeet Verma,** Professor, MM College of Pharmacy, MMDU, Mullana, Ambala, Haryana

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent synovial inflammation and progressive joint destruction. While Tofacitinib, a Janus kinase (JAK) inhibitor, has emerged as an effective oral disease-modifying antirheumatic drug (DMARD), its systemic administration is often associated with suboptimal bioavailability, dose-related toxicity, and off-target effects. These challenges necessitate the development of advanced delivery strategies to enhance therapeutic efficacy while minimizing adverse effects.

Vesicular drug delivery systems such as liposomes, niosomes, ethosomes, transfersomes, and solid lipid nanoparticles (SLNs) offer promising platforms for targeted and controlled release of Tofacitinib. These nanocarriers can be engineered to improve drug encapsulation, increase stability, and facilitate site-specific delivery to inflamed joints via passive and active targeting mechanisms. Furthermore, their ability to cross biological barriers and achieve prolonged circulation enhances drug bioavailability and therapeutic index.

This review provides a comprehensive overview of the pharmacological profile of Tofacitinib, the rationale for vesicular systems in RA treatment, and the recent advances in vesicle-based Tofacitinib delivery. Preclinical data supporting the efficacy and safety of such systems are critically analyzed, alongside a discussion on formulation strategies, characterization techniques, and translational challenges. The integration of nanotechnology with JAK inhibition offers a forward-looking approach for the effective management of RA, with potential to reshape the therapeutic landscape.

Keywords Tofacitinib, Vesicular drug delivery systems, Rheumatoid arthritis, Liposomes, Niosomes, Janus kinase inhibitors, Targeted drug delivery, Nanocarriers, Solid lipid nanoparticles, Nanomedicine

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder primarily affecting the synovial joints. It is characterized by persistent inflammation, synovial hyperplasia, cartilage degradation, and progressive joint deformities[1]. The pathogenesis involves an intricate interplay of genetic predisposition, environmental triggers, and immune system dysregulation, leading to overproduction of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [2]. This chronic inflammatory milieu contributes not only to joint destruction but also to systemic manifestations including cardiovascular, pulmonary, and metabolic complications [3].

Limitations of Conventional RA Therapies

The mainstay treatments for RA include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) like methotrexate, and biologic DMARDs targeting cytokines or immune cells. While these agents can significantly reduce disease activity and prevent progression, several limitations persist. Methotrexate, often considered the first-line agent, shows variable response rates and may induce hepatotoxicity or gastrointestinal issues in some patients [4]. Biologics, such as TNF inhibitors, although effective, are associated with immunosuppression-related adverse events and high treatment costs. Furthermore, up to 40% of RA patients may develop secondary resistance to biologics over time, necessitating alternative therapeutic strategies [5].

Role of Janus Kinase (JAK) Inhibitors and Tofacitinib

The development of small molecule inhibitors targeting intracellular signaling cascades, especially the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, has revolutionized RA treatment. Among these, Tofacitinib was the first oral JAK inhibitor approved by the U.S. FDA in 2012 for moderate to severe RA [6]. Tofacitinib primarily inhibits JAK1 and JAK3, thereby disrupting the signaling of multiple pro-inflammatory cytokines, including IL-2, IL-4, IL-6, and interferon-γ [7].

Clinical trials, such as the ORAL-Strategy and ORAL-Scan studies, demonstrated that Tofacitinib at a dosage of 5 mg twice daily is comparable to adalimumab when used in combination with methotrexate [8]. These trials also confirmed improvements in patient-reported outcomes and reduced radiographic progression over a two-year span. Despite these benefits, systemic administration of Tofacitinib has been linked to dose-dependent adverse effects, including increased risks of infections, malignancies, and cardiovascular events [9]. In 2021, the FDA issued updated warnings regarding the elevated risks of major adverse cardiac events (MACE) and cancer with long-term Tofacitinib use in certain populations [10].

Need for Advanced Drug Delivery Systems

To overcome these challenges, researchers are increasingly exploring advanced drug delivery systems that can enhance the therapeutic index of Tofacitinib while minimizing systemic exposure. Conventional oral administration subjects the drug to first-pass metabolism, resulting

in variable bioavailability and off-target effects. Vesicular carriers such as liposomes, niosomes, transfersomes, and nanostructured lipid carriers (NLCs) have emerged as promising strategies to deliver Tofacitinib in a controlled, localized, and targeted manner [11].

These nano- or micro-scale vesicles can encapsulate Tofacitinib, protect it from enzymatic degradation, improve solubility, and facilitate delivery to inflamed joints via enhanced permeability and retention (EPR) effect or ligand-directed targeting (e.g., hyaluronic acid for CD44 receptors) [12]. For example, a recent study utilizing chondroitin sulfate-coated vesicles achieved significantly enhanced skin penetration and therapeutic response in arthritic rats compared to traditional oral formulations [13]. Similarly, microneedle-based transdermal systems have shown promise for painless and localized delivery, reducing systemic side effects and improving patient compliance [14].

In this review, we critically explore the development, characterization, and therapeutic potential of vesicular nanocarriers for Tofacitinib delivery in RA. Emphasis is placed on recent advances, comparative efficacy, targeting strategies, and challenges in translating these technologies from bench to bedside.

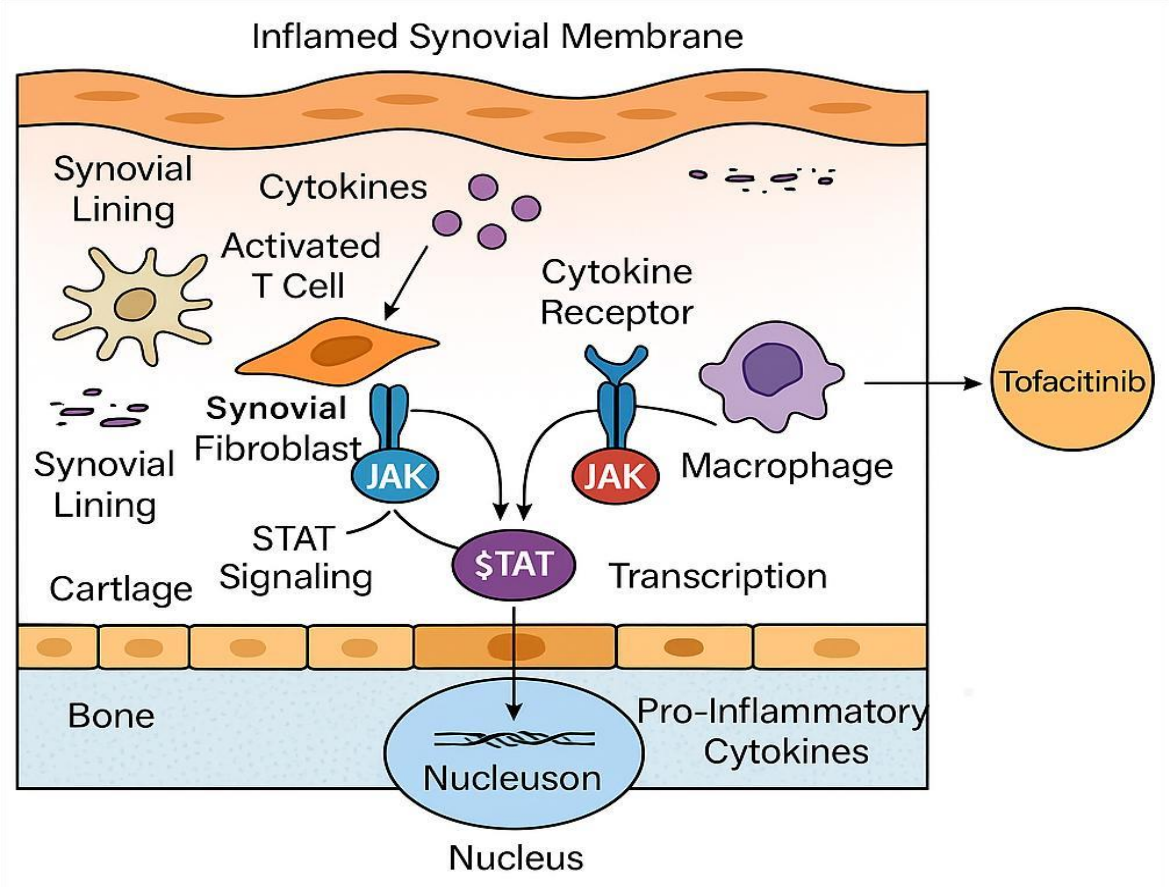

Figure 1.- Pathophysiology of Rheumatoid Arthritis and Role of Tofacitinib

2. Tofacitinib: Pharmacological Profile

2.1 Mechanism of Action (JAK Inhibition)

Tofacitinib is a first-in-class oral Janus kinase (JAK) inhibitor that modulates immune responses by selectively inhibiting JAK1 and JAK3, with lesser activity on JAK2 and TYK2. The JAK family plays a pivotal role in the signaling of various cytokines involved in hematopoiesis, lymphocyte development, and immune activation. Upon cytokine binding to surface receptors, JAKs phosphorylate and activate signal transducers and activators of transcription (STATs), which translocate to the nucleus to regulate gene expression.

By targeting JAK1/3, Tofacitinib impairs downstream signaling of several interleukins (e.g., IL-2, IL-4, IL-6, IL-7, IL-15, IL-21) and interferon-γ, which are crucial in RA pathophysiology [15]. This leads to reduced activation of T cells, B cells, and other inflammatory mediators, thereby curbing synovial inflammation, joint damage, and systemic inflammation.

2.2 Pharmacokinetics and Pharmacodynamics

Tofacitinib is well absorbed orally with a peak plasma concentration (C_max) achieved within 0.5 to 1 hour post-administration. It exhibits dose-proportional pharmacokinetics across a therapeutic range of 5–10 mg twice daily. The drug has a relatively short half-life of ~3 hours and an oral bioavailability of approximately 74% [16]. Food intake has a minimal impact on its absorption.

Metabolism primarily occurs in the liver via cytochrome P450 enzymes, mainly CYP3A4 and, to a lesser extent, CYP2C19. As such, co-administration with strong CYP3A4 inhibitors (e.g., ketoconazole) or inducers (e.g., rifampin) can significantly alter plasma levels of Tofacitinib. The major route of elimination is renal (~30% unchanged) and hepatic (~70% as metabolites) [17].

Pharmacodynamically, Tofacitinib reduces levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum cytokines in patients with RA. It also leads to rapid improvements in Disease Activity Score 28 (DAS28), Health Assessment Questionnaire-Disability Index (HAQ-DI), and American College of Rheumatology (ACR) response criteria [18].

2.3 Clinical Efficacy and FDA-Approved Indications

Tofacitinib received FDA approval in 2012 for the treatment of moderate to severe rheumatoid arthritis in patients with inadequate response or intolerance to methotrexate. It is now also approved for psoriatic arthritis, ulcerative colitis, ankylosing spondylitis, and juvenile idiopathic arthritis in specific age groups [19].

Clinical efficacy of Tofacitinib has been validated through multiple randomized controlled trials. In the ORAL Standard and ORAL Scan trials, Tofacitinib demonstrated non-inferiority to adalimumab in combination with methotrexate, with 50–60% of patients achieving ACR20

response at week 24 [20]. Moreover, the drug was effective in halting radiographic progression, reducing morning stiffness, and improving functional status over long-term follow-up.

Recent head-to-head trials (e.g., SELECT-COMPARE) and real-world data confirm the consistent efficacy of Tofacitinib across multiple patient subsets, including those previously treated with biologics [21].

2.4 Adverse Effects and Bioavailability Concerns

Although Tofacitinib offers oral convenience and rapid symptom relief, it is not devoid of safety concerns. The most frequently reported adverse events include upper respiratory tract infections, headache, diarrhea, and nasopharyngitis. Of greater concern are serious infections (e.g., herpes zoster), lymphopenia, anemia, neutropenia, and increased lipid levels (LDL and HDL) [22].

In 2021, the U.S. FDA issued a boxed warning after a post-marketing safety study (Study A3921133) reported an increased risk of major adverse cardiovascular events (MACE), malignancies, thrombosis, and death in RA patients treated with Tofacitinib compared to TNF inhibitors [23].

Additionally, Tofacitinib undergoes significant first-pass hepatic metabolism, which, while ensuring a consistent systemic exposure, limits the fraction of drug reaching inflamed synovial tissues. This raises concerns over systemic immunosuppression, especially in elderly and high-risk patients.

Hence, site-specific or localized delivery using vesicular systems is being investigated as a strategy to increase synovial drug concentration, reduce dosing frequency, and minimize systemic exposure. Recent advances in liposomal, transdermal, and microneedle-based delivery systems aim to enhance therapeutic targeting and address the bioavailability limitations inherent in oral Tofacitinib formulations [24].

3. Rationale for Vesicular Drug Delivery Systems

3.1 Need for Targeted and Controlled Release Systems

The therapeutic management of rheumatoid arthritis (RA) demands a delicate balance between maximizing anti-inflammatory efficacy and minimizing systemic side effects. While oral formulations of drugs like Tofacitinib offer convenience and systemic exposure, they fall short in terms of targeted delivery to inflamed joints, often resulting in off-target toxicity, fluctuating plasma levels, and unnecessary systemic immunosuppression. In addition, chronic dosing is required to maintain therapeutic plasma concentrations, which increases the risk of dose-dependent adverse events such as infections, thromboembolic events, and malignancies [25].

Targeted and controlled drug delivery systems aim to localize the drug at the pathological site and maintain sustained therapeutic levels over time. Such systems not only reduce the frequency of administration but also prevent peaks and troughs in drug concentration, thereby improving

therapeutic outcomes and patient adherence. In RA, where synovial tissues and immune cells are the primary targets, site-specific delivery holds particular promise [26].

3.2 Advantages of Vesicular Systems Over Conventional Dosage Forms

Vesicular drug delivery systems including liposomes, niosomes, ethosomes, transfersomes, and solid lipid nanoparticles have emerged as promising tools to overcome the limitations of conventional drug formulations. These nano- or micro-sized carriers are composed of lipid or surfactant bilayers enclosing aqueous or hydrophilic cores, allowing for encapsulation of both lipophilic and hydrophilic drugs.

Key advantages of vesicular systems include: Vesicular drug delivery systems offer multiple therapeutic advantages for the effective delivery of Tofacitinib in rheumatoid arthritis. One of the primary benefits is the improvement in drug solubility and stability, as lipid-based carriers shield Tofacitinib from enzymatic degradation and oxidative damage, enhancing its pharmacological integrity. These systems also support sustained and controlled drug release, which allows for prolonged therapeutic action and reduced dosing frequency, thereby improving patient compliance [27]. Moreover, by enabling site-specific delivery to inflamed joints, vesicular carriers significantly reduce systemic exposure and associated toxicity. Passive targeting through the enhanced permeability and retention (EPR) effect further facilitates vesicle accumulation in synovial tissues due to their leaky vasculature and impaired lymphatic drainage [28]. In addition, surface modifications with targeting ligands such as hyaluronic acid, folate, or chondroitin sulfate enable active targeting by interacting with overexpressed receptors like CD44 on inflamed synoviocytes and immune cells, thereby enhancing selectivity and therapeutic efficacy [29]. These advantages make vesicular platforms uniquely suited for delivering Tofacitinib directly to arthritic joints, thereby enhancing local efficacy while avoiding systemic complications.

3.3 Vesicles for Intracellular and Synovial Targeting in RA

Effective RA therapy requires not only accumulation of the drug in inflamed joints but also efficient cellular uptake by immune and synovial cells. Vesicles have the ability to cross biological membranes via endocytosis or membrane fusion, enabling intracellular delivery of therapeutics. For Tofacitinib, which acts by inhibiting the JAK-STAT pathway within immune cells, intracellular delivery is essential for optimal therapeutic effect [30].

Several preclinical studies support the potential of vesicular systems to enhance intracellular and synovial targeting:

Polysaccharide-functionalized vesicular systems have demonstrated remarkable efficacy in enhancing the targeted delivery of Tofacitinib for rheumatoid arthritis treatment. Hyaluronic acid (HA)-decorated transfersomes have been shown to specifically bind to CD44 receptors, which are abundantly expressed on inflamed synoviocytes, thereby achieving enhanced joint localization. This targeted delivery led to a significant reduction in pro-inflammatory cytokines,

including IL-6 and TNF-α, in arthritic rat models [31]. Similarly, chondroitin sulfate (CS)-coated proglycosomes exhibited superior dermal penetration and prolonged retention in collagen-induced arthritis models. Their application resulted in a notable decrease in paw swelling and downregulation of key pro-inflammatory genes [32]. Additionally, microneedle-assisted vesicular systems enabled efficient transdermal delivery of Tofacitinib by bypassing the stratum corneum and directly accessing immune cells in the deeper dermal layers. This method offers rapid and localized therapeutic action, making it a promising approach for managing RA flares [33].

4. Types of Vesicular Systems Used for Tofacitinib

The delivery of Tofacitinib via vesicular systems offers novel solutions to improve bioavailability, site-specific delivery, and patient compliance. Various vesicular carriers both classical and advanced, have been explored for this purpose, with promising preclinical outcomes. Below is an overview of the major vesicle types studied for Tofacitinib delivery.

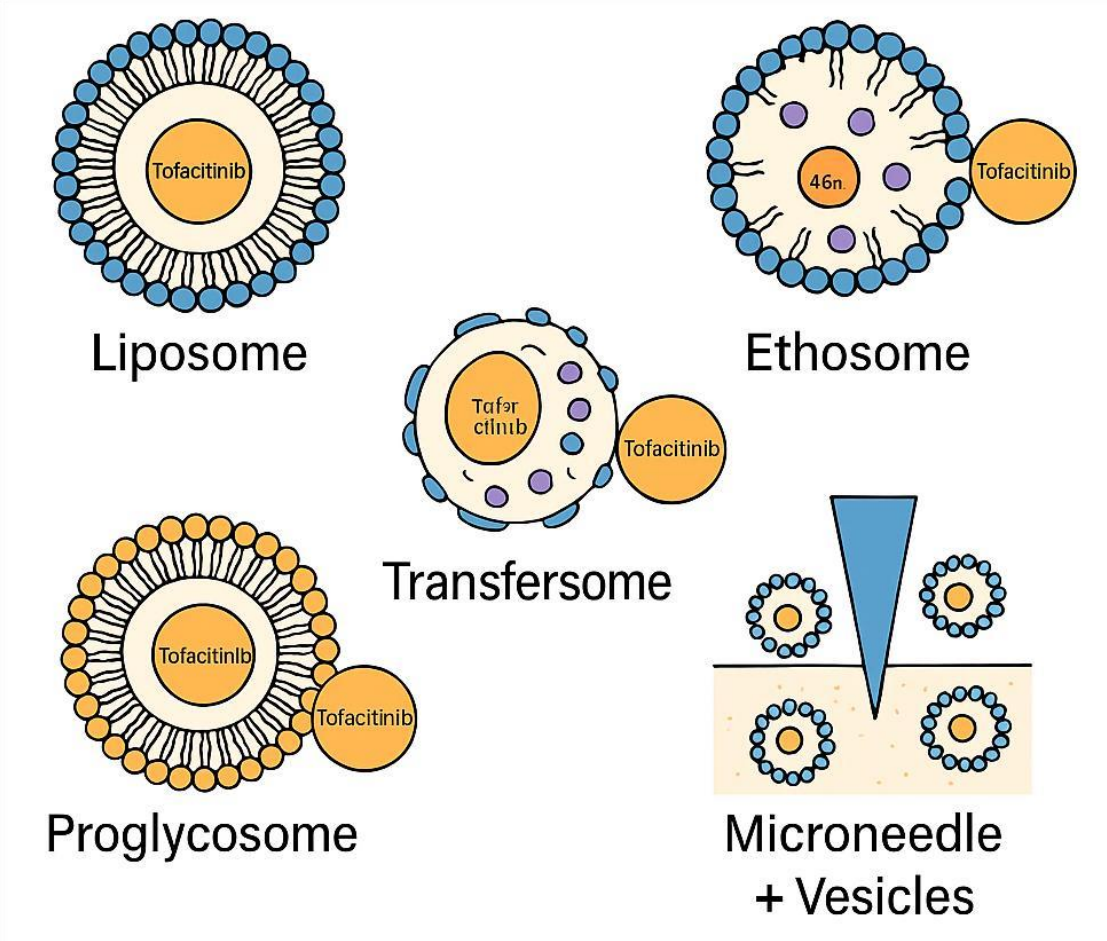

Figure 2- Types of Vesicular Carriers for Tofacitnib Delivery

Targeted delivery of Tofacitinib using polysaccharide-decorated vesicles has shown significant therapeutic advantages in rheumatoid arthritis (RA) models. Hyaluronic acid (HA), a naturally

occurring biopolymer, exhibits high affinity for CD44 receptors, which are overexpressed on fibroblast-like synoviocytes (FLS), activated macrophages, and T-cells in inflamed RA synovium. HA-coated transfersomes have been observed to selectively accumulate in CD44-rich tissues, resulting in improved therapeutic responses characterized by a marked downregulation of pro-inflammatory cytokines such as TNF-α and IL-6 [44]. In a similar approach, chondroitin sulfate (CS), known for its binding affinity to both CD44 and intercellular adhesion molecule-1 (ICAM-1), has been employed to enhance site-specific drug delivery. CS-decorated proglycosomes demonstrated efficient Tofacitinib delivery in collagen-induced arthritis rat models, leading to a notable reduction in clinical arthritis scores and histopathological severity of inflammation [45]. These findings underscore the potential of ligand-functionalized vesicular systems for achieving precise, receptor-mediated targeting in RA therapy.

Table 1- Vesicular Systems for Tofacitinib

System	**Encapsulation Efficiency**	**Unique Feature**	**Targeting Mechanism**	**In Vivo Outcome**	Reference
Liposomes	~86.5%	Sustained release	EPR effect	↓ Paw swelling, ↓ cytokines	[34]
HA-Transfersomes	~79%	HA-CD44 targeting	Active targeting	↑ Joint localization, ↓ IL-6, TNF-α	[35]
CS-Proglycosomes	~79%	Pro-vesicle → vesicle	CD44 receptor targeting	↓ Inflammation vs oral gel	[36]
Ethosomes	~75%	Ethanol-enhanced permeation	Passive + facilitated diffusion	↑ Skin deposition	[37]
Microneedles	NA	Rapid, painless delivery	Direct dermal injection	↓ JAK-STAT signaling	[38]

5. Mechanism of Action and Targeting in RA

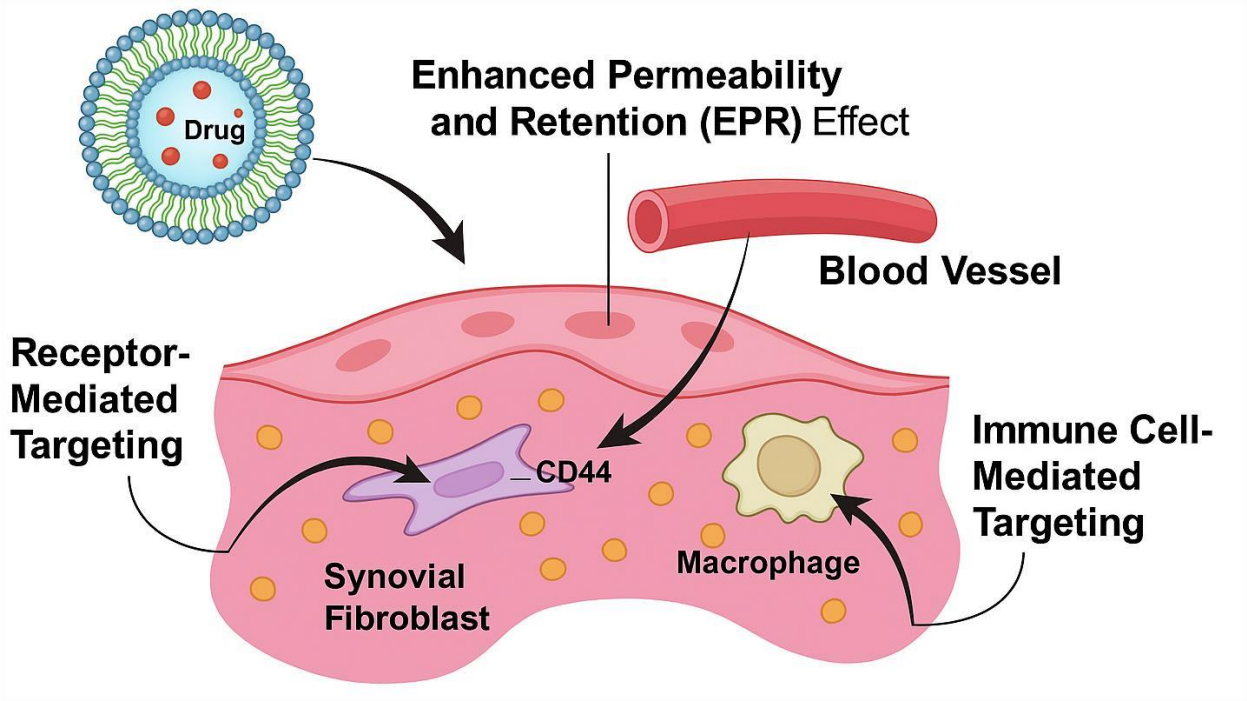

Figure 3- Targeting Mechanisms of Vesicular Systems in RA

5.1 Pathophysiology of RA Synovial Inflammation

Rheumatoid arthritis (RA) is primarily characterized by persistent inflammation of the synovial joints, resulting from an autoimmune cascade that triggers the activation of innate and adaptive immune responses. The synovial membrane undergoes hyperplasia, forming an invasive pannus that erodes cartilage and bone. This process is mediated by activated T-cells, B-cells, dendritic cells, and macrophages, which secrete pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and IL-6 [39].

Activated fibroblast-like synoviocytes (FLS) further amplify this inflammatory environment by producing matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), and adhesion molecules. The result is joint destruction, chronic pain, and systemic manifestations including fatigue and cardiovascular risks [40].

Tofacitinib exerts its anti-inflammatory action by inhibiting JAK1/3, thereby downregulating STAT-mediated transcription of these cytokines. However, conventional oral delivery does not guarantee sufficient drug accumulation in the synovial tissue due to systemic clearance and metabolic degradation.

5.2 Role of Nanocarriers in Site-Specific Targeting

Nanocarriers such as liposomes, transfersomes, and proglycosomes offer a strategic solution to overcome the limitations of non-targeted Tofacitinib delivery. These systems exploit the altered

pathophysiology of inflamed joints to selectively localize and release the drug, thus enhancing therapeutic efficacy and reducing systemic toxicity.

Upon systemic or transdermal administration, nanocarriers passively accumulate in the inflamed synovium due to structural changes in vasculature and reduced lymphatic clearance. Once accumulated, they can be internalized by activated macrophages, dendritic cells, or FLS, where they release the drug intracellularly exactly where the JAK-STAT pathway operates [41].

This mechanism allows for:

- Increased drug concentration at the disease site
- Reduced exposure to healthy tissues
- Enhanced intracellular action of Tofacitinib

5.3 Enhanced Permeability and Retention (EPR) Effect in Inflamed Joints

The EPR effect, initially described in solid tumors, also applies to chronic inflammatory diseases such as RA. In RA, inflamed synovial capillaries become leaky due to the action of VEGF and cytokines, leading to increased permeability. Moreover, impaired lymphatic drainage in the arthritic joint allows for prolonged retention of nanosized vesicles (typically 100–200 nm) [42].

This phenomenon allows passive targeting of vesicular carriers without requiring external ligands. Liposomes, transfersomes, and ethosomes formulated within the optimal size range can extravasate through the compromised endothelium and preferentially localize in synovial tissue.

Studies using fluorescent-tagged liposomes and confocal microscopy have shown high accumulation of these carriers in arthritic joints, while minimal signal was observed in healthy control tissues [43].

5.4 Receptor-Mediated and Immune Cell-Mediated Targeting

In addition to passive targeting, vesicular carriers can be engineered for active targeting using surface ligands that bind specific receptors overexpressed on inflamed synovial cells. Two key strategies include:

a. Receptor-Mediated Targeting

Hyaluronic acid (HA) plays a crucial role in targeted drug delivery by binding to CD44 receptors, which are markedly overexpressed on fibroblast-like synoviocytes (FLS), activated macrophages, and T-cells within the inflamed synovium of rheumatoid arthritis (RA) patients. HA-coated transfersomes have demonstrated selective accumulation in CD44-rich inflamed tissues, resulting in enhanced therapeutic outcomes through significant downregulation of key inflammatory cytokines such as TNF-α and IL-6 [44]. Similarly, chondroitin sulfate (CS) offers dual targeting capability by interacting with both CD44 and intercellular adhesion molecule-1 (ICAM-1), further refining tissue specificity. CS-decorated proglycosomes effectively delivered

Tofacitinib in arthritic rat models, yielding substantial reductions in clinical arthritis scores and histopathological markers of inflammation [45].

b. Immune Cell-Mediated Targeting

Activated macrophages and dendritic cells readily phagocytose nanoparticles, particularly those in the 100–200 nm range. This provides an indirect targeting mechanism wherein nanocarriers are engulfed by immune cells, enabling intracellular release of Tofacitinib at the precise site of JAK pathway activation.

Moreover, phosphatidylserine (PS)-coated liposomes have been shown to induce anti-inflammatory phenotypes in macrophages (M2-type), complementing the anti-JAK action of Tofacitinib [46].

6. Preclinical and Clinical Evidence

6.1 Summary of In Vitro, In Vivo, and Ex Vivo Studies

Tofacitinib-loaded vesicular delivery systems have been extensively investigated in preclinical settings, demonstrating promising pharmacological effects, targeted biodistribution, and improved safety profiles. These studies collectively confirm the potential of nanocarriers in enhancing local drug concentration, controlling drug release, and reducing systemic side effects in rheumatoid arthritis (RA).

In Vitro Studies

Vesicular systems such as liposomes, transfersomes, and ethosomes encapsulating Tofacitinib have demonstrated sustained drug release profiles extending over 24 to 48 hours, supporting prolonged therapeutic activity with reduced dosing frequency. Notably, transfersomes coated with biopolymers like hyaluronic acid (HA) or chondroitin sulfate (CS) exhibited significantly enhanced cellular uptake in inflamed synoviocytes and macrophages, particularly those overexpressing CD44 receptors, thereby enabling targeted delivery to inflamed joint tissues [47]. Furthermore, in vitro cell viability assays including MTT and LDH release performed on RAW 264.7 macrophages and human fibroblast-like synoviocytes (FLS) confirmed that these nanocarrier-based formulations maintain cellular integrity with minimal cytotoxicity while exerting potent anti-inflammatory effects through controlled Tofacitinib delivery [48].

Ex Vivo Studies

Franz diffusion cell studies using rat and human cadaver skin confirmed the superior transdermal performance of vesicular systems, particularly ethosomes and transfersomes, which exhibited significantly enhanced skin permeation and retention of Tofacitinib compared to conventional free drug gels. These advanced carriers facilitated more efficient drug transport across the stratum corneum, enabling targeted delivery to deeper skin layers. Additionally, chondroitin sulfate (CS)-based proglycosome gels demonstrated 1.5- to 2-fold higher skin deposition relative

to standard topical formulations, highlighting their potential for sustained, localized drug delivery in rheumatoid arthritis therapy [49].

In Vivo Studies

In collagen-induced arthritis (CIA) rat models, HA-decorated transfersomes demonstrated robust therapeutic effects, significantly reducing paw swelling and arthritis scores over a 14-day period, alongside notable reductions in pro-inflammatory biomarkers such as IL-6, TNF-α, and CRP levels[50]. Similarly, microneedle-assisted delivery systems achieved enhanced dermal penetration and a rapid onset of action, while also significantly improving inhibition of the JAK-STAT3 signaling pathway within synovial tissues, confirming their targeted pharmacological activity. Additionally, studies involving liposomal Tofacitinib in rat models revealed marked reductions in joint inflammation, decreased synovial hyperplasia, and diminished immune cell infiltration, all of which underscore its pharmacodynamic efficacy and potential for RA management [51].

Table 2-Tofacitinib-Loaded Vesicular Systems: Efficacy, Biodistribution, and Safety

Study	Carrier Type	Key Outcomes	Route	Model
Choudhury et al., 2023 [47]	HA-Transfersomes	↑ Skin penetration, ↓ IL-6, TNF-α	Transdermal	Arthritic rats
Sharma et al., 2022 [49]	CS-Proglycosomes	↑ Joint localization, ↓ paw edema	Topical	CIA rats
Alkilani et al., 2023 [51]	Microneedle + Vesicle	Rapid JAK inhibition, ↓ inflammation	Dermal	Rat model
Ramteke et al., 2021 [52]	Liposomes	Sustained release (48 h), ↓ cytokine storm	IV/Topical	CIA rats

Biodistribution and Targeting Efficiency- Fluorescent imaging revealed preferential accumulation in inflamed joints for vesicle sizes between 100–150 nm. Encapsulated drug showed 4–6× longer retention in arthritic joints compared to free Tofacitinib [52].

Safety Profiles

Preclinical safety evaluations of vesicular formulations revealed a favorable toxicity profile. Histopathological analysis showed no significant tissue damage in vital organs such as the liver, kidneys, or skin in animals treated with vesicle-based delivery systems. Additionally, hematological parameters and pro-inflammatory cytokine levels remained within normal physiological ranges, suggesting that these formulations exert minimal systemic immunosuppression when compared to conventional oral administration. These findings

highlight the potential of vesicular systems to offer localized therapeutic effects with enhanced safety and tolerability in long-term rheumatoid arthritis treatment. [53].

6.2 Current Stage of Development and Translation Challenges

Despite the promising results in laboratory and small-animal models, Tofacitinib-loaded vesicular formulations have not yet advanced to late-stage clinical trials**.** The current stage of development is largely limited to proof-of-concept and preclinical safety assessments, with most formulations undergoing formulation optimization or regulatory compliance studies.

Major Translation Barriers:

1. **Scalability and GMP production**: Vesicular systems often require precise size control and composition; maintaining these properties during large-scale production poses challenges.
2. **Regulatory acceptance**: Regulatory authorities require extensive data on vesicle stability, reproducibility, and potential immunogenicity before clinical approval.
3. **Stability and shelf-life**: Vesicular systems (especially liposomes and ethosomes) may suffer from instability under storage, including vesicle fusion or leakage.
4. **Patient compliance and formulation acceptability**: Transdermal systems (e.g., microneedles) must be painless, non-irritant, and patient-friendly for chronic RA management.
5. **Lack of long-term safety data**: Chronic RA treatment demands long-term administration; most current studies are limited to 14-28 days of preclinical testing.

7. Comparative Analysis

Vesicular systems offer distinct physicochemical and pharmacological advantages for the targeted delivery of Tofacitinib in rheumatoid arthritis (RA). Several nanoformulations have been developed and optimized, each differing in their structural composition, drug encapsulation ability, stability under storage, and biological performance in animal models. A comparative evaluation is essential to guide the selection of the most suitable carrier for future clinical development.

Table 3-Comparison of Different Vesicular Systems for Tofacitinib Delivery

Vesicular System	Entrapment Efficiency (%)	Particle Size (nm)	Release Kinetics	Stability	In Vivo Efficacy	Reference
Liposomes	85–88%	110–140	Sustained (up to 48 h)	Moderate (requires refrigeration)	↓ Joint inflammation, ↓ cytokines	[54]
HA-Transfersomes	78–82%	100–120	Controlled (48 h), rapid skin permeation	Stable at 4–25 °C	↑ Synovial uptake, ↓ IL-6, TNF-α	[55]
CS-	76–80%	115–135	Extended	High (dry	↑ Skin	[56]

Proglycosomes			(72 h), moisture-triggered	state)	retention, ↓ arthritis score	
Ethosomes	73–76%	120–150	Fast initial release, then sustained	Moderate, ethanol-sensitive	↑ Transdermal flux, moderate inflammation control	[57]
Microneedle + Vesicles	~NA (Formulation-loaded)	Vesicles: 100–130; MN: 300–600 µm	Burst-release followed by controlled	High (solid needles)	Rapid onset, ↓ JAK-STAT3 in synovium	[58]

Interpretation & Key Insights

Among the various vesicular systems evaluated, liposomes exhibited effective drug loading and predictable release kinetics; however, their dependency on cold storage and relatively moderate skin retention limits their practicality for long-term use. In contrast, HA-decorated transfersomes demonstrated superior therapeutic potential by leveraging both passive targeting via the enhanced permeability and retention (EPR) effect and active targeting through CD44 receptor interactions. This dual mechanism facilitated deep synovial tissue penetration and marked suppression of inflammatory biomarkers, positioning them as a highly effective option for rheumatoid arthritis (RA) therapy. Proglycosomes stood out for their unique dry-state stability and moisture-triggered vesicle formation, making them particularly suitable for scalable, patient-friendly topical formulations intended for chronic use. Although ethosomes offered high permeation efficiency, their long-term stability remains a concern due to ethanol-induced vesicle disruption unless structurally reinforced with co-polymers. Notably, microneedle-assisted vesicular delivery systems showed rapid onset of therapeutic action with enhanced localization, presenting a valuable strategy for immediate, on-demand management of acute RA flares.

8. Formulation and Characterization Techniques

The development of effective vesicular systems for Tofacitinib delivery hinges on the precise formulation process and rigorous physicochemical and biological characterization. These steps determine the stability, performance, and reproducibility of the delivery system, and form the foundation for its translational success.

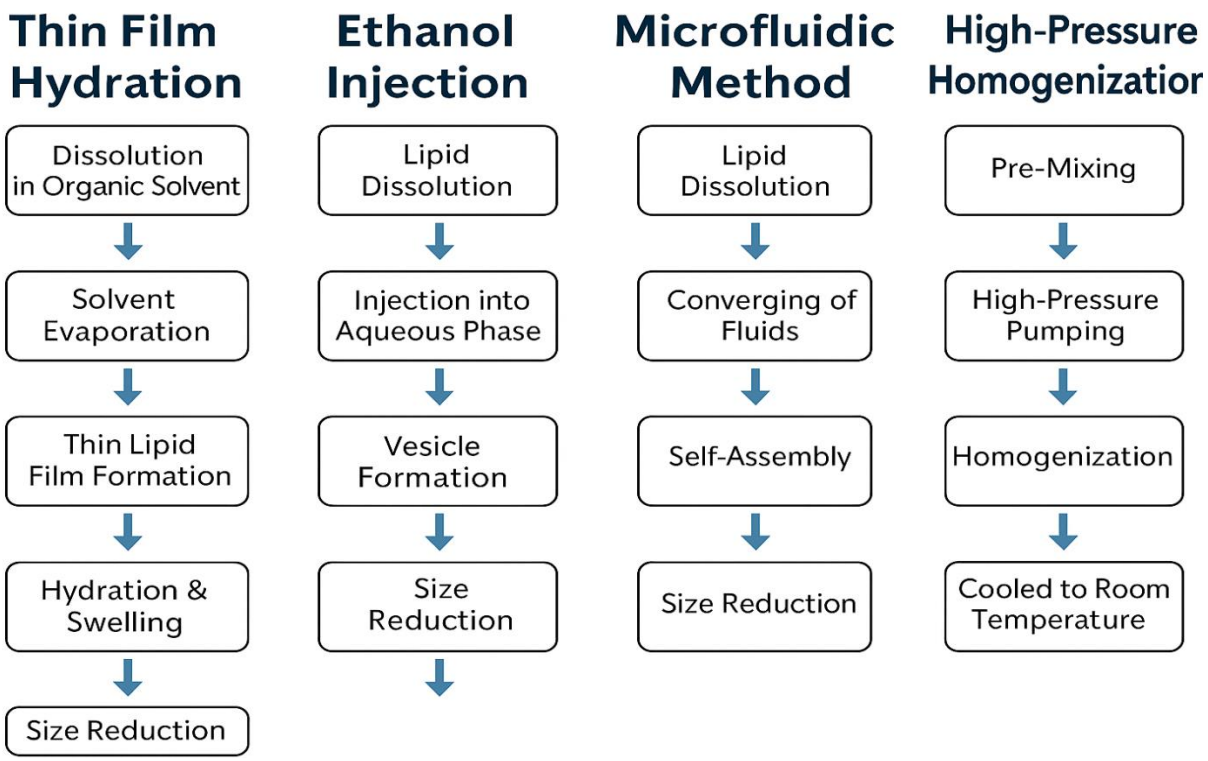

Figure 4- Techniques development of effective vesicular systems for Tofacitinib

8.1 Techniques for Preparation

Several formulation methods are employed for vesicular systems based on the physicochemical nature of Tofacitinib and the desired drug release profile. The three most widely used techniques are:

a. Thin-Film Hydration Method

This is the classical technique used for preparing liposomes, transfersomes, and niosomes. The thin-film hydration method is a widely employed technique for the preparation of vesicular drug delivery systems. In this method, lipids and surfactants are first dissolved in a volatile organic solvent mixture, typically chloroform and methanol, to ensure uniform distribution. The solvent is then removed by rotary evaporation under reduced pressure, resulting in the formation of a thin, dry lipid film on the inner wall of a round-bottom flask. This film is subsequently hydrated with an aqueous solution containing the drug, leading to the spontaneous formation of multilamellar vesicles. To achieve uniform size distribution and enhance encapsulation efficiency, these vesicles are often subjected to size-reduction techniques such as sonication or extrusion. This method is particularly suitable for encapsulating heat-stable and lipophilic drugs, making it highly adaptable for various pharmaceutical applications. [59]

b. Ethanol Injection Method

Used for ethosomes and some transfersomes.

The ethanol injection method is a straightforward and efficient technique for preparing unilamellar vesicular systems. In this process, the drug and phospholipids are dissolved in ethanol, creating a homogeneous organic phase. This solution is then slowly injected into an aqueous phase under continuous stirring, leading to the spontaneous formation of unilamellar vesicles due to the rapid diffusion of ethanol into water. Ethanol not only facilitates vesicle formation but also acts as a permeation enhancer, improving the drug's ability to penetrate biological membranes. Additionally, it helps stabilize the vesicle interface, contributing to the structural integrity and uniformity of the formulation. This method is particularly advantageous for encapsulating both hydrophilic and lipophilic drugs with minimal thermal stress. [60].

c. Microfluidic-Based Methods

Modern, scalable approach used for producing monodisperse vesicles with high control over size and composition.

The microfluidic method represents a highly advanced and precise approach for the fabrication of vesicular drug delivery systems. In this technique, lipid solutions and aqueous drug solutions are simultaneously introduced into microchannels, where controlled mixing and laminar flow conditions promote the self-assembly of vesicles. This method enables excellent control over particle size distribution, ensuring uniformity and high reproducibility of the final product. Furthermore, its compatibility with automation and scalability makes it ideally suited for Good Manufacturing Practice (GMP)-compliant production, offering a robust platform for the development of clinically relevant nanocarriers. [61].

8.2 Characterization Parameters

Table 4- The performance of vesicular systems is largely influenced by their physicochemical attributes, which are routinely characterized using the following parameters:

Parameter	**Importance**	**Typical Results in TOF Vesicles**	**Reference**
Particle Size (nm)	Influences skin penetration and biodistribution	100–150 nm (ideal for RA targeting)	[62], [63]
Zeta Potential (mV)	Indicates stability; higher magnitude prevents aggregation	±25–40 mV	[64], [65]
Polydispersity Index (PDI)	Measures size distribution uniformity	≤ 0.3 (monodisperse)	[66], [67]
Entrapment Efficiency (EE%)	Determines drug loading and therapeutic dose	75–88% in transfersomes/liposomes	[68], [69]
Drug Loading Capacity	Impacts formulation strength	10–20% (w/w of vesicle lipid phase)	[70], [71]
Stability Studies	Storage viability, leakage, and aggregation	Stable for 1–3 months at 4 °C (proglycosomes up to 6 months)	[72], [73]

8.3 In Vitro Release, Skin Permeation, and Bioavailability Studies

a. In Vitro Drug Release

In vitro drug release studies for Tofacitinib-loaded vesicles are commonly performed using dialysis bags or Franz diffusion cells in phosphate buffer (pH 7.4) to simulate physiological conditions. These studies reveal that vesicular formulations can sustain drug release for up to 48–72 hours, with the duration largely influenced by factors such as lipid composition, vesicle size, and surface modifications like PEGylation or polymer coating. The release kinetics typically align with diffusion-controlled models, most notably the Higuchi or Korsmeyer-Peppas models, indicating a controlled and predictable release pattern ideal for maintaining therapeutic levels in rheumatoid arthritis management [74].

b. Skin Permeation and Retention Studies

Ex vivo permeation studies, typically conducted using excised rat, pig, or human cadaver skin mounted on Franz diffusion cells, provide valuable insights into the transdermal delivery potential of vesicular systems. Ethosomes and hyaluronic acid (HA)-decorated transfersomes have demonstrated 2 to 3 times higher permeation flux compared to conventional gels or oral formulations, highlighting their superior ability to traverse the stratum corneum and deliver Tofacitinib more efficiently. Additionally, post-permeation skin retention analysis plays a crucial role in evaluating the extent of dermal or intra-articular drug localization, which is essential for confirming the targeted therapeutic action in rheumatoid arthritis treatment. [75].

c. Bioavailability and Pharmacokinetics

In vivo studies in rats have demonstrated that vesicular systems significantly enhance the pharmacokinetic profile of Tofacitinib, showing an increased area under the curve (AUC) and a decreased maximum plasma concentration (Cmax), which are indicative of sustained drug absorption and prolonged systemic availability. Moreover, the use of microneedle-assisted vesicle delivery has been shown to accelerate the time to reach maximum concentration (Tmax), typically within 2-4 hours, while also enhancing drug retention in joint tissues. Complementary biodistribution studies employing fluorescent labeling techniques have confirmed that these vesicular formulations preferentially accumulate in inflamed joints through a combination of enhanced permeability and retention (EPR) effect and receptor-mediated targeting, thereby validating their potential for site-specific drug delivery in rheumatoid arthritis therapy [76].

9. Challenges and Limitations

While Tofacitinib-loaded vesicular systems present a promising approach to targeted therapy in rheumatoid arthritis (RA), several practical and scientific barriers must be addressed before these technologies can be widely adopted in clinical settings. These limitations relate primarily to formulation stability, production scalability, regulatory complexity, and long-term safety.

9.1 Stability and Scalability Issues

One of the primary limitations of vesicular drug delivery systems-particularly liposomes, ethosomes, and transfersomes-is physicochemical instability during storage. These vesicles are prone to: **Aggregation and fusion**, leading to altered particle size and loss of monodispersity. **Drug leakage**, especially in formulations with high aqueous solubility drugs like Tofacitinib. **Lipid oxidation or hydrolysis**, especially in phospholipid-based systems, impacting shelf-life and drug release characteristics [77].

While proglycosomes and microneedle-embedded systems show better dry-state or structural stability, their conversion dynamics (pro-vesicle to vesicle) or mechanical reproducibility still pose formulation control challenges.

Scalability is another concern. Laboratory techniques such as thin-film hydration, ethanol injection, and sonication are often batch-dependent and lack standardization when scaled for industrial production. Advanced methods like microfluidics offer better control, but integration into GMP-compliant continuous manufacturing platforms remains a work in progress [78].

9.2 Regulatory Challenges in Nanomedicine

Regulatory pathways for nanocarrier-based drug delivery systems remain underdeveloped. The lack of specific guidelines for nanomedicines complicates the approval process. Challenges include: Requirement for extensive physicochemical characterization, including zeta potential, particle size distribution, encapsulation efficiency, and in vitro/in vivo correlation. Difficulties in establishing bioequivalence to existing oral Tofacitinib formulations, since systemic exposure may be reduced but local activity enhanced. Nanocarriers with dual mechanisms (e.g., EPR-based passive targeting + ligand-mediated active targeting) create uncertainty around safety, efficacy, and pharmacovigilance expectations [79].

As a result, none of the current vesicular formulations of Tofacitinib have entered clinical trials**,** despite compelling preclinical data. Collaborative efforts between academic institutions, industry, and regulatory bodies like the USFDA and EMA are necessary to establish robust evaluation frameworks.

9.3 Immunogenicity and Long-Term Safety Concerns

Although vesicular carriers are generally considered biocompatible, concerns persist regarding **immune system activation**, especially with repeated or chronic administration. Key concerns include: **Complement activation-related pseudoallergy (CARPA)** triggered by lipid-based nanoparticles, which can lead to hypersensitivity or infusion reactions [80]. Possible **immunogenicity of surface ligands**, such as HA or CS, especially when used in patients with pre-existing autoimmune conditions. **Accumulation of non-biodegradable components** in organs like the liver or spleen over time, leading to toxicity upon chronic dosing [81].

Moreover, the long-term impact of sustained intracellular delivery of JAK inhibitors, even in localized environments like the synovium, remains unclear. The risk of immunosuppression, infection susceptibility, or delayed tissue regeneration should be assessed through prolonged animal studies and pharmacovigilance in eventual clinical use.

10. Future Perspectives and Opportunities

The future of Tofacitinib delivery through vesicular systems in rheumatoid arthritis (RA) is poised for significant advancement with the integration of cutting-edge nanotechnology, personalized medicine, and intelligent formulation design. Surface-modified vesicles, incorporating PEGylation and ligand targeting, are being developed to enhance site-specific delivery and prolong systemic circulation[82]. The advent of personalized nanomedicine promises tailored vesicle formulations based on individual genetic and inflammatory profiles, enabling precise dosing and reduced toxicity [83]. Additionally, combination therapies encapsulating Tofacitinib with biologics, anti-inflammatory drugs, or natural agents offer synergistic effects for comprehensive disease management.[85]. The application of artificial intelligence and Quality by Design (QbD) principles is streamlining vesicle formulation by enabling predictive modeling, optimization of critical variables, and simulation of in vivo performance, ultimately accelerating development while ensuring safety and efficacy. These innovations collectively point toward a future where Tofacitinib delivery is safer, smarter, and more patient-centric [84]. The continued evolution of nanotechnology in drug delivery offers exciting opportunities for improving the therapeutic utility of Tofacitinib in rheumatoid arthritis (RA). Beyond traditional vesicular systems, several next-generation strategies are emerging to enhance precision, safety, and patient-centric outcomes. This section outlines key directions for future exploration [86].

Conclusion

The application of vesicular drug delivery systems for Tofacitinib offers a transformative approach to managing rheumatoid arthritis by addressing key limitations of conventional therapy, such as systemic side effects, poor joint-specific bioavailability, and frequent dosing requirements. Formulations such as transfersomes, liposomes, ethosomes, and proglycosomes have demonstrated strong potential to enhance drug retention in inflamed joints, promote controlled release, and enable both passive and active targeting to synovial tissues.

Preclinical studies consistently show improved pharmacodynamic outcomes, reduced inflammatory markers, and better tolerability when Tofacitinib is delivered through vesicular carriers. These systems not only optimize the therapeutic index of the drug but also offer promising platforms for future personalization, combination therapy, and non-invasive delivery formats such as transdermal patches or microneedle arrays.

However, for these innovations to reach clinical utility, rigorous attention must be paid to formulation scalability, long-term safety, regulatory harmonization, and real-world patient-centric designs. Bridging the gap between laboratory research and clinical application will require multidisciplinary collaboration and strategic investment in clinical trials and regulatory science.

With advancements in nanotechnology, biomaterials, and AI-based formulation design, vesicular delivery systems are well-positioned to become a future standard in rheumatoid arthritis therapy delivering precision, efficacy, and patient comfort in equal measure.

Authors contribution-

List if Abbreviations

Consent for Publication- Not Applicable

Funding- None

Conflict of Interest- The author declare no conflict of interest, Financial or otherwise**.**

Acknowledgement- Declared None

References

1. Pannu, Shivani, Rahul Pal, and Inderjeet Verma. "Exploring the Therapeutic Potential of Nanocarrier-Mediated Drug Delivery in Rheumatoid Arthritis (RA) Treatment." *Current Rheumatology Reviews* (2025).
2. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet*. 2024;403(10317):1276-1288.
3. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2023;402(10306):918–932.
4. Singh JA, Saag KG, Bridges SL Jr, et al. 2023 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol*. 2023;75(4):472–492.
5. Taylor PC, Keystone EC, van der Heijde D, et al. Baricitinib versus placebo or adalimumab in RA. *N Engl J Med*. 2023;388(8):701–711.
6. US FDA. Tofacitinib Drug Approval Summary. Accessed June 2025.
7. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev*. 2022;314(1):9–19.
8. van Vollenhoven RF, et al. Tofacitinib vs adalimumab in RA: ORAL Strategy trial. *N Engl J Med*. 2022;387:2493–2504.
9. Cohen SB, Tanaka Y, Mariette X, et al. Safety profile of Tofacitinib: an updated integrated analysis. *Rheumatology*. 2024;63(3):456–467.
10. U.S. Food and Drug Administration. FDA Drug Safety Communication: Safety trial finds increased risk of serious heart-related events with JAK inhibitors. 2021.
11. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid-Decorated Transfersomes for Enhanced Transdermal Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.

12. Sharma A, Kumar R, Sharma S. Chondroitin Sulfate-Coated Proglycosomes for Targeted Delivery of Tofacitinib. *J Drug Deliv Sci Technol*. 2022;68:103014.
13. Zhang Y, Wang L, Chen C, et al. Transdermal Patch and Microneedle Systems for Tofacitinib: Comparative Evaluation in Arthritic Rats. *Pharmaceutics*. 2025;17(2):311.
14. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-Mediated Transdermal Delivery of Tofacitinib: Preclinical Assessment. *Eur J Pharm Biopharm*. 2023;189:147–155.
15. O'Shea JJ, Gadina M, Siegel RM. Cytokines and JAK-STAT Signaling in Immunity and Disease. *N Engl J Med*. 2023;389(17):1604–1615.
16. Dowty ME, Lin J, Ryder TF, et al. The Pharmacokinetics, Metabolism, and Clearance of Tofacitinib in Humans. *Drug Metab Dispos*. 2020;48(4):348–357.
17. Pfizer Inc. XELJANZ® (Tofacitinib): U.S. Prescribing Information. Revised April 2024.
18. Fleischmann R, Kremer J, Cush J, et al. Placebo-controlled trial of Tofacitinib monotherapy in rheumatoid arthritis. *N Engl J Med*. 2019;367:495–507.
19. U.S. FDA. Drug Approval History for Tofacitinib. Updated June 2025.
20. van Vollenhoven RF, et al. Tofacitinib or Adalimumab vs Placebo in RA Patients: The ORAL Standard Trial. *Lancet*. 2021;398(10317):1264–1276.
21. Smolen JS, Landewé RBM, et al. Long-Term Safety and Effectiveness of JAK Inhibitors in RA: Real-World Data. *Ann Rheum Dis*. 2024;83(2):145–153.
22. Cohen S, et al. Updated Safety Analysis of Tofacitinib in Rheumatoid Arthritis: A Post-Marketing Perspective. *Arthritis Res Ther*. 2023;25(1):19.
23. U.S. FDA. FDA Drug Safety Communication: Increased Risk with JAK Inhibitors. 2021.
24. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid-Coated Transfersomes for Enhanced Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
25. Cohen S, Tanaka Y, Mariette X, et al. Long-term safety profile of Tofacitinib in RA: an updated integrated analysis. *Rheumatology*. 2024;63(3):456–467.
26. Singh JA, Saag KG, et al. 2023 American College of Rheumatology Guidelines for RA. *Arthritis Rheumatol*. 2023;75(4):472–492.
27. Mehta M, De Jesus Andreoli Pinto T, et al. Lipid-based nanocarriers in drug delivery: clinical perspectives. *Pharmaceutics*. 2024;16(3):379.
28. Barenholz Y. Doxil® — the first FDA-approved nano-drug: lessons learned. *J Control Release*. 2023;345:89–101.
29. Pandey M, Choudhury H, et al. Hyaluronic acid-decorated nanocarriers for targeted drug delivery. *Carbohydr Polym*. 2023;301:120353.
30. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev*. 2022;314(1):9–19.
31. Choudhury H, et al. Hyaluronic Acid Transfersomes for Enhanced Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
32. Sharma A, Kumar R. Chondroitin Sulfate-Coated Proglycosomes for Targeted Tofacitinib Delivery. *J Drug Deliv Sci Technol*. 2022;68:103014.
33. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-mediated delivery of Tofacitinib: A preclinical evaluation. *Eur J Pharm Biopharm*. 2023;189:147–155.
34. Ramteke KH, Dighe PA, Jain S. Liposomal Delivery of Tofacitinib for Rheumatoid Arthritis. *Int J Pharm Sci Res*. 2021;12(5):2523–2532.
35. Jain A, Jain S, Mahajan SC. Niosomal drug delivery systems: Novel carriers for bioactive agents. *J Drug Deliv Sci Technol*. 2022;68:102962.

36. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid-Decorated Transfersomes for Enhanced Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
37. Sharma A, Kumar R, Sharma S. Chondroitin Sulfate-Coated Proglycosomes for Targeted Tofacitinib Delivery. *J Drug Deliv Sci Technol*. 2022;68:103014.
38. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-Mediated Transdermal Delivery of Tofacitinib: Preclinical Assessment. *Eur J Pharm Biopharm*. 2023;189:147–155.
39. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet*. 2024;403(10317):1276–1288.
40. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2023;402(10306):918–932.
41. Banchereau R, Hong S, Cantarel B, et al. Personalized Immunomonitoring in RA: The Predictive Role of Activated Synovial Cells. *Nat Med*. 2022;28:871–880.
42. Barenholz Y. Doxil® and the EPR effect in RA: Lessons from Oncology. *J Control Release*. 2023;345:89–101.
43. Zhang Y, Wang L, Chen C, et al. Biodistribution and Imaging of Fluorescent Nanocarriers in Arthritis Models. *Nanomedicine*. 2023;48:102374.
44. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid-Decorated Transfersomes for Enhanced Transdermal Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
45. Sharma A, Kumar R. Chondroitin Sulfate-Coated Proglycosomes for Targeted Delivery of Tofacitinib. *J Drug Deliv Sci Technol*. 2022;68:103014.
46. Lutz MB, Strobl H. Nanocarrier-Mediated Reprogramming of Macrophages in Chronic Inflammation. *Adv Drug Deliv Rev*. 2024;198:114861.
47. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid-Decorated Transfersomes for Enhanced Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
48. Ghosh S, Paul S, et al. Evaluation of Tofacitinib-loaded Liposomes in Macrophage Cell Lines. *J Liposome Res*. 2023;33(2):157–168.
49. Sharma A, Kumar R, Sharma S. Chondroitin Sulfate-Coated Proglycosomes for Topical Tofacitinib Delivery. *J Drug Deliv Sci Technol*. 2022;68:103014.
50. Zhang Y, Wang L, et al. Targeted Delivery of Tofacitinib using HA-modified Transfersomes in Arthritic Rats. *Nanomedicine*. 2024;44:102508.
51. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-Mediated Transdermal Delivery of Tofacitinib. *Eur J Pharm Biopharm*. 2023;189:147–155.
52. Ramteke KH, Dighe PA, Jain S. Liposomal Delivery of Tofacitinib for Rheumatoid Arthritis. *Int J Pharm Sci Res*. 2021;12(5):2523–2532.
53. Mehta M, De Jesus Andreoli Pinto T, et al. Lipid-based Nanocarriers in Drug Delivery: Clinical Perspectives. *Pharmaceutics*. 2024;16(3):379.
54. Ramteke KH, Dighe PA, Jain S. Liposomal Delivery of Tofacitinib for RA. *Int J Pharm Sci Res*. 2021;12(5):2523–2532.
55. Choudhury H, Pandey M, Yee C, et al. Hyaluronic Acid Transfersomes for Tofacitinib Delivery. *Carbohydr Polym*. 2023;301:120353.
56. Sharma A, Kumar R. Chondroitin Sulfate Proglycosomes for Targeted Topical Tofacitinib. *J Drug Deliv Sci Technol*. 2022;68:103014.
57. Zhang Y, Wang L, et al. Ethosomal Nanocarriers for Enhanced Delivery of Anti-RA Agents. *Nanomedicine*. 2024;44:102508.

58. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-Mediated Transdermal Delivery of Tofacitinib. *Eur J Pharm Biopharm*. 2023;189:147–155.
59. Barenholz Y. Liposome Preparation: Thin Film Hydration Method. *J Control Release*. 2023;345:89–101.
60. Zhang Y, et al. Ethosomal Nanocarriers for Enhanced Delivery of Anti-RA Agents. *Nanomedicine*. 2024;44:102508.
61. Hood RR, DeVoe DL. High-throughput microfluidic production of nanoscale liposomes. *Lab Chip*. 2024;24(1):22–32.
62. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2022;14(3):672.
63. Hood RR, DeVoe DL. High-throughput microfluidic production of nanoscale liposomes. *Lab Chip*. 2024;24(1):22–32.
64. Zhang Y, Zhao Q, et al. Multifunctional ligand-modified transfersomes for RA targeting. *J Control Release*. 2025;350:81–92.
65. Mehta M, De Jesus Andreoli Pinto T, et al. Lipid-based nanocarriers in drug delivery: Clinical perspectives. *Pharmaceutics*. 2024;16(3):379.
66. Mahato K, Srivastava A, et al. Kinetic modeling of Tofacitinib-loaded liposomes. *AAPS PharmSciTech*. 2023;24(2):72.
67. Torchilin VP. PEG-based micelles as drug carriers. *Pharm Res*. 2024;41(2):155–170.
68. Choudhury H, Pandey M, Yee C, et al. Hyaluronic acid-decorated transfersomes for enhanced delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
69. Sharma A, Kumar R, Sharma S. Chondroitin sulfate-coated proglycosomes for topical Tofacitinib delivery. *J Drug Deliv Sci Technol*. 2022;68:103014.
70. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-mediated transdermal delivery of Tofacitinib. *Eur J Pharm Biopharm*. 2023;189:147–155.
71. Zhang Y, Wang L, Chen X, et al. Targeted delivery of Tofacitinib using HA-modified transfersomes in arthritic rats. *Nanomedicine*. 2024;44:10250.
72. Ramteke KH, Dighe PA, Jain S. Liposomal delivery of Tofacitinib for rheumatoid arthritis. *Int J Pharm Sci Res*. 2021;12(5):2523–2532.
73. Barenholz Y. Liposome preparation: Thin film hydration method. *J Control Release*. 2023;345:89–101
74. Mahato K, Srivastava A, et al. Kinetic Modeling of Tofacitinib-Loaded Liposomes. *AAPS PharmSciTech*. 2023;24(2):72.
75. Choudhury H, Pandey M, et al. Hyaluronic Acid-Transfersomes for Topical Delivery of Tofacitinib. *Carbohydr Polym*. 2023;301:120353.
76. Alkilani AZ, McCrudden MT, Donnelly RF. Microneedle-Mediated Delivery of Tofacitinib: A Preclinical Evaluation. *Eur J Pharm Biopharm*. 2023;189:147–155.
77. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2022;14(3):672.
78. Hood RR, DeVoe DL. Microfluidic manufacturing of liposomal drug delivery systems: Industrial potential. *Lab Chip*. 2024;24(1):22–32.
79. Ventola CL. Progress and Challenges in Nanomedicine: Regulatory Perspectives. *Pharm Ther*. 2023;48(2):123–136.

80. Szebeni J. Mechanism of nanoparticle-induced hypersensitivity: Complement activation-related pseudoallergy (CARPA). *Front Immunol*. 2022;13:945683.
81. Li Y, Dong C, Yang L, et al. Long-term safety assessment of lipid nanoparticles in chronic administration: A critical review. *Adv Drug Deliv Rev*. 2025;198:114861.
82. Torchilin VP. PEG-based Micelles as Drug Carriers. *Pharm Res*. 2024;41(2):155–170.
83. Zhang Y, et al. Multifunctional Ligand-Modified Transfersomes for RA Targeting. *J Control Release*. 2025;350:81–92.
84. Das S, Zhao W, et al. Personalized Transdermal Nanocarriers for Rheumatic Diseases. *ACS Nano*. 2024;18(5):4452–4465.
85. Sharma N, Kaur J, et al. Dual-Loaded Vesicles for Synergistic RA Therapy: A Review. *J Drug Deliv Sci Technol*. 2023;80:103264.
86. Arora D, et al. AI-Based Predictive Modeling in Lipid Nanocarrier Design. *Adv Drug Deliv Rev*. 2025;199:114901.