

Journal of Molecular Science

www.jmolecularsci.com

ISSN:1000-9035

Formulation And Evaluation Of Sustained Release Tofacitinib-Loaded Hydrogel Beads In The Treatment Of Rheumatoid Arthritis

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Article Information

Received: 02-06-2025

Revised: 20-06-2025

Accepted: 09-07-2025

Published:21-07-2025

Keywords*Rheumatoid arthritis, hydrogels, polymers, treatment, drug delivery, joints.***ABSTRACT**

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation, joint destruction, and progressive disability. Despite advancements in therapeutic agents, conventional treatments face challenges such as systemic toxicity, limited bioavailability, and poor patient compliance. Hydrogel-based drug delivery systems have emerged as promising platforms for localized and sustained drug release in RA management. Hydrogels possess unique properties, including high water content, biocompatibility, and tunable mechanical strength, enabling targeted delivery of anti-inflammatory agents, disease-modifying drugs, and biologics directly to affected joints. These systems minimize systemic exposure and reduce side effects, enhancing therapeutic efficacy. Preclinical studies have demonstrated that hydrogel formulations effectively reduce inflammation, inhibit pannus formation, and promote cartilage regeneration in animal models of RA. Various hydrogels, including natural polymers (e.g., alginate, chitosan) and synthetic polymers (e.g., polyethylene glycol, polyvinyl alcohol), have been investigated for their ability to encapsulate drugs, peptides, and nanoparticles. Advances in stimuli-responsive hydrogels further enable on-demand drug release triggered by environmental changes such as pH, temperature, or enzymes. Moreover, injectable hydrogels simplify administration and ensure joint-specific retention, addressing challenges related to frequent dosing. Future research should focus on optimizing hydrogel formulations to enhance mechanical stability, biodegradability, and bioactivity while ensuring regulatory compliance and scalability. Overall, hydrogel-based systems hold immense potential to revolutionize RA treatment by providing safer, more effective, and patient-friendly therapeutic options.

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1. INTRODUCTION

Rheumatoid arthritis (RA) is classified as a chronic pathology that inflicts damage upon human joints due to an autoimmune dysfunction. The immune system initiates the destruction of its own cells, and as a result of this autoimmune response, certain cytokines are released, including interleukins (such as IL-6 and IL-7), leukotrienes, and other inflammatory mediators like tumor necrosis factors (TNF- α), which culminate in the degradation of biological and physicochemical structures [1]. The

average incidence rate of RA among adult's ranges from 0.5% to 1%, with annual reports of 5 to 50 new cases per 100,000 individuals, particularly among the elderly or female populations within certain demographic groups [2]. RA is distinguished by persistent synovitis, angiogenesis, and immunological responses that progressively inflict damage upon joints, bones, and cartilage. This presents a comprehensive depiction of the pathogenesis and related inflammatory mechanisms in the RA-affected joint in contrast to a healthy joint [3]. Autoantibodies can be identified in the affected joints and serum of patients to evaluate rheumatoid factors and citrullinated peptides. As much as 50% of the risk associated with the development of RA can be attributed to genetic factors, whereas smoking represents another significant risk factor contributing to RA prevalence in developed nations. This disease is disproportionately prevalent among female populations compared to male and elderly groups. Active RA frequently remains unmanageable, leading to debilitating chronic inflammation, tissue damage, instability, and deformity [4].

1. **Pathology of Rheumatoid Arthritis**

2. Numerous theories have been proposed, despite the fact that the pathophysiological mechanisms underlying RA remain incompletely understood [5]. According to reports, immunological processes might take place years before joint inflammation symptoms appear; this is known as the "pre-RA phase". Modified self-antigens, including as immunoglobulin G (IgG), type 2 collagen, and vimentin, might result from the interplay between environmental influences and epigenetic changes on the genomic structure [6]. Citrullination is a post-translational alteration that occurs when peptidyl arginine deiminases transform these proteins with arginine residues into citrulline [63,64]. Furthermore, cytokine production from joint conditions such as synovial hyperplasia or infections can result in joint inflammation and altered self-antigens [7]. The immune system can no longer identify citrullinated proteins (vimentin, type II collagen, histones, fibrin, fibronectin, Epstein-Barr nuclear antigen 1, α -enolase) as self-structures because of the susceptibility genes HLA-DR1 and HLA-DR4 [8]. Antigen-presenting cells (APCs) are activated dendritic cells that take up antigens in order to trigger an immune response. The entire complex moves to the lymph node, where CD4+ helper T cell activation occurs [9]. B cells in the lymph node's germinal center are stimulated by sequential and reciprocal impulses with T cells, a process known as costimulation in immunology. The relationship between CD28 and CD80/86 is an illustration of costimulation. At this stage, B cells develop into plasma cells that make autoantibodies

based on the receptors of the precursor cells, undergo somatic hypermutation, or class-switch recombination, and begin to multiply [10]. Self-tissues and organs are unintentionally targeted by autoantibodies, which are proteins generated by an immune system that is no longer able to distinguish between self and non-self-components. The most researched autoantibodies implicated with RA include RF and ACPA [11]. RF is an IgM antibody that targets the Fc component of IgG, also known as the constant region, and has an 85% testing specificity in RA patients. Air pollution, which includes a variety of gases (such as carbon monoxide, sulfur dioxide, nitrates, and ozone) and suspended particle matter (PM) of different sizes, has recently drawn more attention in the field of RA [12]. Numerous man-made and natural processes, including as the burning of fossil fuels, the chemical industry, the use of solvents, volcanic eruptions, wind-blown dust, emissions from plants, and agriculture, can discharge pollutants into the atmosphere [13]. Most often, the clinical effects of air pollution are examined in connection with respiratory conditions. Ozone has been shown to harm the alveoli, a vital component of the respiratory system that filters carbon dioxide and oxygen. By reacting with various enzymes, pollutants can also inflict secondary damage on lung tissue, leading to infection or inflammation of the lungs [14]. Research on RA has increasingly emphasized environmental determinants, particularly air pollution. Air pollution is constituted by a complex amalgamation of chemical agents, including carbon monoxide, sulfur dioxide, nitrates, ozone, and various sizes of suspended particulate matter (PM). A plethora of anthropogenic and natural origins, such as agricultural practices, fossil fuel combustion, the chemical sector, solvent usage, volcanic activity, aeolian dust, and biogenic emissions, contribute to the release of these hazardous substances into the atmosphere [15]. Empirical studies have indicated a contributory role of air pollution in the pathophysiological mechanisms underlying RA, despite its predominant association with respiratory disorders. Pollutants inflict damage upon the alveoli, pivotal structures within the respiratory system that facilitate the exchange of carbon dioxide and oxygen. Ozone has been evidenced to compromise alveolar integrity and instigate pulmonary inflammation or infection through its interaction with enzymatic components in lung tissues [16]. A series of epidemiological studies carried out in the United States, Canada, and Sweden have elucidated a correlation between airborne contaminants and the incidence of RA [17]. In one particular investigation, nitrates and sulfur dioxide were delineated as significant risk factors for the onset of RA. Another research

endeavor conducted in Verona, Italy, encompassing a cohort of 888 RA patients, demonstrated an association between air pollution and elevated levels of C-reactive protein (CRP), augmented severity of RA, and diminished responsiveness to biological therapeutic interventions. Mechanistically, air pollutants facilitate the pathogenesis of RA through multiple biological pathways [18]. Reactive oxygen species (ROS) generated via the inhalation of particulate matter activate nuclear factor kappa B (NF- κ B), inciting T helper cell type 1 (Th1) responses [19]. Th1 cells secrete pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines foster the maturation of monocytes into dendritic cells, which subsequently present autoantigens to self-reactive T lymphocytes [20]. The activated T lymphocytes traverse to targeted tissues, precipitating joint inflammation and erosion. Citrullination, a post-translational modification process, is likewise catalyzed by ROS. In this particular biochemical phenomenon, arginine residues within proteins undergo conversion to citrulline, culminating in the synthesis of citrullinated peptides [21]. These peptides incite an autoimmune response, resulting in the production of anti-citrullinated protein antibodies (ACPAs). ACPAs interact with Fc receptors on immune cells and activate the complement cascade, thereby inciting joint inflammation and osseous erosion [22]. Another environmental variable that impacts the pathogenesis of RA is diminished exposure to ultraviolet B (UVB) radiation, which subsequently decreases the production of 1,25-dihydroxyvitamin D3 in the epidermis. Vitamin D3 functions as an immunomodulator through the activation of vitamin D receptors (VDR). Suboptimal vitamin D levels can impair immunomodulatory capabilities, potentially serving as a trigger for RA [23]. The gut microbiota, representing the most densely populated bacterial consortium within the human organism, is another pivotal factor implicated in the pathogenesis of RA [24]. Intestinal dysbiosis, characterized by an imbalance in the composition of gut microbial populations, may instigate autoimmune processes, such as the activation of antigen-presenting cells (APCs) via toll-like receptors (TLRs) or nod-like receptors (NLRs) [25]. Dysbiosis is also associated with alterations in intestinal permeability, the facilitation of T cell differentiation, and the exacerbation of mucosal inflammation [26]. Research utilizing 16S rRNA sequencing and metagenomic shotgun sequencing has unveiled compositional discrepancies in gut microbiota between RA patients and healthy individuals [27]. Specific bacterial taxa, including *Prevotella copri*, *Collinsella*, and *Lactobacillus salivarius*, exhibit increased abundance in RA

patients, whereas genera such as *Bacteroides*, *Faecalibacterium*, *Veillonella*, and *Haemophilus* demonstrate reduced prevalence [28].

3. Hydrogels Delivery System

4. Hydrogels (table 1), defined by their distinctive three-dimensional architectures of hydrophilic polymers, have emerged as a pivotal element in the evolution of biomaterial science, fundamentally transforming applications across a diverse array of biomedical disciplines [28]. These architectures, possessing the capacity to absorb and retain substantial quantities of water, are recognized for their extraordinary ability to expand without disintegration, preserving structural stability through various chemical or physical cross-linking methodologies. They can be classified into natural, synthetic, and hybrid hydrogels based on their origin and composition [29]. Natural hydrogels are derived from biomaterials such as alginate, chitosan, and hyaluronic acid, offering excellent biocompatibility but may have limited mechanical strength. Synthetic hydrogels, including polyvinyl alcohol (PVA) and polyethylene glycol (PEG), provide greater mechanical and chemical stability [30]. Hybrid hydrogels combine natural and synthetic components, optimizing the benefits of both types. The properties of hydrogels make them highly suitable for drug delivery systems. Their high-water content provides a moist environment, which is essential for sustaining drug activity and improving biocompatibility [31]. Hydrogels exhibit tunable swelling and degradation characteristics, allowing controlled and sustained release of therapeutic agents. The porosity of the hydrogel network can be adjusted to regulate drug loading and release rates, enabling precise dosage control [32]. Moreover, hydrogels can respond to physiological stimuli such as pH, temperature, and enzymes, making them ideal for targeted and responsive drug delivery (figure 2). For instance, pH-responsive hydrogels can release drugs specifically in inflamed or diseased tissues where pH levels differ from normal physiological conditions [33]. Temperature-sensitive hydrogels can undergo sol-to-gel transitions, providing site-specific delivery with minimal invasiveness. These properties ensure hydrogels can deliver a wide range of therapeutic agents, from small-molecule drugs to proteins and nucleic acids. The advantages of hydrogel-based drug delivery systems extend beyond their structural and functional versatility [34]. Hydrogels offer localized and sustained drug release, minimizing systemic side effects and enhancing therapeutic efficacy. Their biocompatibility and biodegradability reduce the risk of immune responses and toxicity. Injectable hydrogels enable minimally invasive administration, improving patient compliance and comfort [35]. Additionally, hydrogels can

encapsulate drugs without compromising their activity, protecting sensitive molecules from degradation and enhancing bioavailability. The ability to incorporate targeting ligands and bioactive molecules further improves specificity and therapeutic outcomes [36]. Hydrogels can also be designed to mimic natural tissues, supporting cell adhesion and proliferation, which is particularly useful for regenerative medicine applications. Overall, hydrogels represent a promising platform for advanced drug delivery strategies, addressing the limitations of conventional therapies and improving treatment outcomes for diseases like RA [37]. The mechanism of drug release from hydrogels depends on the structural characteristics of the hydrogel matrix, the nature of the encapsulated drug, and environmental conditions. Hydrogels can facilitate sustained and prolonged drug release, minimizing systemic toxicity and enhancing therapeutic efficacy [38]. The network structure of hydrogels allows for drug diffusion, swelling-induced release, or enzymatic degradation, offering flexibility for designing delivery systems that meet specific therapeutic requirements. The key mechanisms involved in hydrogel-based drug delivery include controlled release systems, stimuli-responsive behaviors, and biodegradable properties, which collectively provide precision and adaptability in drug administration [38]. Controlled release mechanisms in hydrogels are primarily based on diffusion, swelling, and degradation. Diffusion-controlled release involves the movement of drug molecules through the hydrogel matrix driven by concentration gradients. This mechanism is suitable for hydrophilic drugs, where the release rate is governed by the porosity and mesh size of the hydrogel network. In swelling-controlled release, the hydrogel absorbs water and expands, allowing the encapsulated drug to diffuse out [39]. The swelling behavior depends on the polymer composition and cross-linking density, enabling tunable drug release profiles. Degradation-controlled release relies on the breakdown of hydrogel matrices via hydrolysis or enzymatic action, leading to gradual drug release. Biodegradable hydrogels degrade into non-toxic byproducts, making them suitable for temporary implants or localized therapy [40]. By combining these mechanisms, hydrogels can achieve zero-order or near-zero-order drug release kinetics, ensuring consistent therapeutic levels over extended durations. Stimuli-responsive hydrogels, also known as smart hydrogels, exhibit dynamic behavior in response to external or internal stimuli such as pH, temperature, light, magnetic fields, or specific biomolecules [41]. These hydrogels are designed to undergo structural or phase transitions that trigger drug release upon stimulation. pH-

responsive hydrogels are particularly useful for targeting specific regions of the body, such as the stomach or intestines, where pH variations occur [42]. For example, hydrogels with acidic or basic functional groups can swell or shrink at specific pH levels, enabling site-specific drug delivery. Temperature-sensitive hydrogels, composed of polymers like poly(N-isopropylacrylamide), respond to changes in temperature by undergoing sol-gel transitions. Such systems can release drugs at physiological temperatures, making them suitable for injectable formulations [43]. Light-sensitive hydrogels utilize photodegradable linkages or photoisomerizable groups, enabling spatial and temporal control over drug release through light irradiation. Magnetic and electric field-responsive hydrogels can release drugs upon exposure to electromagnetic fields, offering remote-controlled delivery systems [44]. These stimuli-responsive mechanisms provide precise control over drug release, making hydrogels highly versatile for applications in cancer therapy, wound healing, and tissue engineering. Biodegradable hydrogels are designed to break down naturally within the body, eliminating the need for surgical removal and reducing the risk of long-term complications [45]. These hydrogels are synthesized from biodegradable polymers such as poly(lactic acid), poly(glycolic acid), and natural polymers like chitosan, collagen, and gelatin. The degradation process is typically triggered by hydrolysis, enzymatic activity, or oxidative reactions, resulting in the gradual release of encapsulated drugs. Biodegradable hydrogels are particularly advantageous for localized and site-specific delivery, as they can provide sustained drug release over time and then degrade into harmless byproducts [46]. For instance, enzymatically degradable hydrogels can respond to the presence of specific enzymes overexpressed in diseased tissues, enabling targeted therapy. Moreover, biodegradable hydrogels can be engineered to match the degradation rate with the desired drug release profile, ensuring optimal therapeutic outcomes. These systems are widely applied in post-surgical drug delivery, tissue regeneration, and anti-cancer therapies. Their biocompatibility and degradation characteristics make them suitable for applications in sensitive biological environments, where long-term biostability is not required [47]. Polysaccharide-based hydrogels have gained significant attention in the field of biomedical engineering and drug delivery due to their natural abundance, biocompatibility, and biodegradability [48]. Derived from natural sources such as plants, algae, and microbial fermentation, polysaccharides offer a versatile platform for hydrogel development. These hydrogels can form physically or chemically cross-

linked networks, providing tunable mechanical and swelling properties [49]. Common polysaccharides used in hydrogel synthesis include alginate, chitosan, hyaluronic acid, and dextran. Their hydrophilic nature allows for high water retention, making them ideal candidates for drug encapsulation and release applications. Alginate-based hydrogels are widely used due to their ionotropic gelation properties [50]. When exposed to divalent cations such as calcium ions, alginate forms a stable hydrogel network that can encapsulate bioactive molecules and release them in a controlled manner. These hydrogels are pH-sensitive, enabling targeted drug release in specific regions of the gastrointestinal tract. Similarly, chitosan-based hydrogels, derived from chitin, exhibit antimicrobial properties and mucoadhesiveness, enhancing their application in wound healing and mucosal drug delivery [51]. Chitosan hydrogels can respond to pH changes, allowing site-specific drug delivery in acidic environments such as tumors. Hyaluronic acid-based hydrogels are known for their biocompatibility and ability to interact with cellular receptors, making them suitable for tissue engineering and regenerative medicine. These hydrogels can support cell proliferation and migration, promoting tissue repair. Dextran-based hydrogels, on the other hand, offer oxidative degradability, making them effective for reactive oxygen species-responsive drug delivery systems [52]. Polysaccharide hydrogels can also be modified with functional groups to enhance their mechanical strength and responsiveness to stimuli. The drug release mechanism in polysaccharide-based hydrogels is governed by diffusion, swelling, and degradation [53]. For example, hydrogels synthesized from alginate and chitosan exhibit pH- and ion-responsive behavior, providing site-specific and sustained drug release. Polysaccharide-based hydrogels can be integrated with nanoparticles or bioactive agents to enhance their therapeutic efficacy and targeting ability. Their biodegradability ensures safe elimination from the body, minimizing adverse effects [54]. Polysaccharide-based hydrogels are widely used in diverse biomedical applications, including wound dressings, tissue engineering scaffolds, and injectable drug delivery systems. Their ability to form gels under mild conditions and encapsulate sensitive biomolecules like proteins and nucleic acids makes them highly suitable for advanced drug delivery technologies [55]. The drug release mechanism in polysaccharide-based hydrogels is governed by diffusion, swelling, and degradation. For example, hydrogels synthesized from alginate and chitosan exhibit pH- and ion-responsive behavior, providing site-specific and sustained drug release. Additionally, polysaccharide-based

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5. Types of Drugs Delivered Through Hydrogel Systems

Hydrogels, characterized by their hydrophilic three-dimensional network structures, have garnered significant attention as drug delivery systems owing to their biocompatibility, tunable properties, and capacity to encapsulate a wide range of therapeutic agents [71]. These systems offer controlled and sustained drug release profiles, making them ideal candidates for various drug delivery applications (table 2). This section explores the diverse categories of drugs delivered through hydrogel systems, including non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biologics and monoclonal antibodies, and herbal and natural compounds [72]. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed to manage pain, inflammation, and fever associated with various medical conditions, including arthritis, postoperative recovery, and musculoskeletal injuries. Despite their therapeutic efficacy, the prolonged use of NSAIDs often leads to gastrointestinal complications, renal impairment, and cardiovascular risks [73]. To mitigate these adverse effects, hydrogel systems have emerged as a promising delivery platform for NSAIDs. Hydrogels provide sustained drug release, thereby maintaining therapeutic concentrations of NSAIDs at the target site for an extended duration and reducing systemic exposure. For instance, hydrogels loaded with ibuprofen, naproxen, or diclofenac have demonstrated enhanced bioavailability and localized delivery, minimizing systemic toxicity [74]. Furthermore, thermosensitive hydrogels enable site-specific drug release in response to temperature fluctuations, ensuring precise drug administration for inflammation-related disorders. The use of biodegradable and pH-sensitive hydrogels further

augments the potential for targeted NSAID delivery, reducing dosing frequency and enhancing patient compliance [75]. Disease-modifying anti-rheumatic drugs (DMARDs) represent a class of pharmacological agents designed to alter the course of inflammatory and autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Conventional administration of DMARDs, including methotrexate, sulfasalazine, and hydroxychloroquine, is often associated with systemic toxicity, low bioavailability, and poor patient adherence. Hydrogel systems offer an innovative approach to overcoming these limitations by facilitating localized and controlled delivery of DMARDs. Hydrogels can encapsulate DMARDs within their polymeric network, enabling gradual drug release and prolonged therapeutic effects. Injectable hydrogels, in particular, have gained prominence for intra-articular delivery, targeting inflamed joints while sparing healthy tissues [76]. Stimuli-responsive hydrogels can be engineered to release DMARDs in response to changes in pH, temperature, or enzymatic activity within diseased tissues, thereby enhancing treatment precision. Recent advancements in hydrogel formulations incorporating nanocarriers have further improved DMARD stability, bioavailability, and cellular uptake, offering new avenues for effective management of autoimmune disorders. Biologics and monoclonal antibodies have revolutionized the treatment landscape for chronic and autoimmune diseases, including rheumatoid arthritis, psoriasis, and cancer. These therapeutic agents, derived from living cells, exhibit high specificity and potency but pose challenges related to stability, immunogenicity, and delivery [67]. Hydrogels serve as ideal carriers for biologics and monoclonal antibodies due to their ability to protect sensitive biomolecules from degradation while enabling controlled release. Injectable hydrogel systems have been developed to deliver biologics, such as tumor necrosis factor (TNF)-alpha inhibitors and interleukin (IL) antagonists, directly to inflamed or cancerous tissues. By maintaining localized drug concentrations, hydrogels minimize systemic side effects and improve treatment efficacy [68]. Moreover, hydrogels can be designed with bioresponsive properties, allowing drug release to be triggered by enzymatic or pH changes in the microenvironment. Advances in hydrogel fabrication techniques, including hybrid hydrogels incorporating nanoparticles or liposomes, have further enhanced the stability and bioactivity of biologics, paving the way for more efficient drug delivery strategies. The integration of herbal and natural compounds into hydrogel systems has garnered significant interest in recent years, driven by the growing demand for alternative and

complementary therapies [69]. Herbal and natural compounds, such as curcumin, quercetin, resveratrol, and ursolic acid, possess anti-inflammatory, antioxidant, and anticancer properties but face challenges related to poor solubility, low bioavailability, and rapid metabolism. Hydrogel systems address these limitations by providing a hydrophilic matrix that enhances solubility and prolongs drug release. For instance, curcumin-loaded hydrogels have demonstrated sustained anti-inflammatory and wound-healing effects, making them suitable for topical and transdermal applications. Similarly, hydrogels incorporating resveratrol have shown promise in anticancer therapy by enabling targeted delivery to tumor sites and reducing systemic toxicity [70].

Application of Hydrogels in RA Management

Hydrogels have emerged as promising therapeutic platforms for the management of RA due to their ability to deliver drugs in a controlled and targeted manner. Their biocompatible and tunable properties make them suitable for addressing the chronic inflammation, joint damage, and pain associated with RA [78]. This section explores key applications of hydrogels in RA management (table 3), focusing on intra-articular drug delivery, transdermal drug delivery, and injectable hydrogels. Intra-articular drug delivery involves the direct injection of therapeutic agents into the synovial cavity of affected joints, providing localized treatment while minimizing systemic side effects. Hydrogels have shown significant potential in this application due to their ability to sustain drug release and maintain therapeutic concentrations within the joint space [79]. The use of hydrogels for intra-articular delivery of anti-inflammatory agents, DMARDs, and biologics has demonstrated improved outcomes in reducing inflammation, alleviating pain, and preventing cartilage degradation. Thermosensitive hydrogels that gel upon injection and respond to temperature changes have further enhanced the precision and convenience of intra-articular drug delivery [80]. Stimuli-responsive hydrogels that release drugs in response to pH or enzyme activity within inflamed joints provide targeted treatment, reducing the frequency of injections and enhancing patient compliance. Transdermal drug delivery systems utilizing hydrogels offer a non-invasive alternative for RA treatment, enabling sustained and controlled drug release through the skin. Hydrogel-based patches and gels loaded with NSAIDs, DMARDs, or herbal compounds provide localized treatment, reducing systemic exposure and minimizing adverse effects [81]. These systems are particularly beneficial for patients who experience gastrointestinal intolerance or prefer non-oral delivery routes. Hydrogels enhance the

permeability of drugs through the skin by maintaining a hydrated environment, improving drug solubility, and acting as reservoirs for sustained release. Recent advancements in microneedle-integrated hydrogels have further improved transdermal delivery efficiency, enabling deeper penetration of therapeutic agents into inflamed tissues [82]. Such systems offer convenience and improved adherence, making them suitable for long-term RA management. Injectable hydrogels have emerged as a versatile approach for RA treatment, offering minimally invasive drug delivery options with the ability to fill irregular joint spaces and conform to tissue architecture. These hydrogels can encapsulate a variety of drugs, including DMARDs, biologics, and anti-inflammatory agents, and provide sustained release at the target site. Injectable hydrogels can be designed to respond to specific stimuli, such as enzymes, pH, or temperature, ensuring on-demand drug release in inflamed joints. Their ability to deliver cells and growth factors has also opened new avenues for cartilage regeneration and joint repair in RA patients [83]. Furthermore, injectable hydrogels can serve as scaffolds for tissue engineering applications, promoting tissue regeneration while delivering therapeutic agents. This dual functionality positions injectable hydrogels as a transformative approach to RA treatment, addressing both symptom management and tissue repair [84].

Hydrogels as artificial extracellular matrices for dynamic *in vitro* models

The process of obtaining therapeutic approval is lengthy, from *in vitro* experiments to widespread clinical application. With the rising costs of drug development, the practice of using animal testing as a reliable predictor of human outcomes has been questioned. Animal models are considered the gold standard in preclinical research for studying the pathophysiology of RA [91]. They exhibit numerous similarities to human arthritic conditions and are commonly used to test new treatment methods. However, animal models have limitations, such as incomplete development of arthritis, differences in the pathophysiology of animals compared to humans, and their inadequacy for extensive drug screening. After reviewing several systematic reviews that assessed the results of animal testing, many publications over the past two decades have highlighted the drawbacks of animal experimentation. Recently, a group of academics and industry specialists proposed a potential alternative for the near future: human-based microphysiological models, developed through tissue engineering principles [92]. These systems benefit from using human cells, resolving the issue of species-related translation discrepancies. However, they present significant engineering

challenges, particularly concerning the complexity of organs and systemic dynamics. A biomimetic dynamic articular cartilage model must include biocompatible hydrogels, with physiologically relevant physical and biochemical signals for chondrogenesis; multi-axial mechanical forces, simulating the movement of joints; and the physiological shear stress of synovial fluid, which transports nutrients and regulates soluble oxygen levels [93]. To enhance drug development outcomes, existing models with higher throughput and scalability must be developed in parallel with these advancements. The production and accumulation of hyaline cartilage-specific extracellular matrix, leading to neotissues with improved biochemical properties resembling native tissue. Another study explored the effect of interstitial flow gradients on chondrocyte-seeded agarose hydrogels in a bioreactor [94]. The results indicated that flow stimulation of chondrocyte-seeded agarose hydrogels enhances glycosaminoglycan and type II collagen deposition on the hydrogel surface exposed to flow. Additionally, it was found that interstitial flow improves convective mass transport, regardless of molecular size, near the hydrogel surface, and that the convective transport effect diminishes with depth in relation to the flow gradients [95]. A study by Daly et al. examined whether dynamic bioreactor culture, under specific oxygen conditions, could expedite the development of large cartilage tissues using mesenchymal stem cell-loaded alginate hydrogels. The dynamic culture conditions were tested at 20% and 3% oxygen levels. At 20% oxygen, dynamic culture significantly reduced chondrogenesis in engineered tissues of all sizes. However, at 3% oxygen, dynamic culture markedly increased the distribution and quantity of cartilage matrix components, collagen II, and sulfated glycosaminoglycan compared to static conditions [96].

Preclinical and Clinical Consideration

The selection of animal models and defect creation methods should align with the clinical claims for cartilage repair devices and reflect cartilage pathophysiology along with biomechanical conditions. Animal models can be classified into small (e.g., rodents and rabbits) and large (e.g., goats, sheep, pigs, dogs, and horses) categories [97]. Small animal models are typically used for proof-of-concept studies, evaluating biocompatibility, safety, and material optimization. In contrast, large animal models are essential for translational studies to validate device performance under regulatory guidelines. The anatomical features such as cartilage thickness, defect dimensions, and joint load-bearing capacity should mimic human anatomy [98]. Device performance

must also be evaluated in diseased states to predict efficacy under pathological conditions. Skeletally mature animals are preferred to avoid spontaneous cartilage regeneration, which is prominent in younger animals. Partial-thickness defects should retain the calcified cartilage layer to prevent healing through non-chondrocytic mechanisms. Unilateral models are advantageous for studies involving weight-bearing conditions, while bilateral models allow intra-animal comparisons [99]. Defects should preferably be created in load-bearing regions to closely replicate clinical scenarios. Short-term (6–12 weeks) studies evaluate biocompatibility and early integration, whereas long-term (6–12 months) studies assess cartilage repair, matrix maturation, and device stability. Preclinical validation must follow GLP standards to ensure traceability and reproducibility [100]. Standard Operating Procedures (SOPs) for device preparation, surgical techniques, and characterization must be documented. While non-GLP studies may reduce costs during early development, GLP-compliant pivotal studies are mandatory for regulatory approval. Useful for initial feasibility studies, evaluating biocompatibility, biodegradation, and safety. However, their thin cartilage layer and intrinsic healing ability limit their application for articular defect studies. Frequently used due to larger joint sizes and early skeletal maturity. Critical defect sizes (>3 mm) are needed to minimize spontaneous healing. Load-bearing defects should be prioritized over trochlear groove defects to better simulate human conditions. Suitable for osteochondral defect studies. Low self-healing potential and exposed stifle joints enable macroscopic evaluation and arthroscopy. Ethical concerns and limited availability of skeletally mature dogs pose challenges. Most commonly used due to appropriate cartilage thickness and joint anatomy [101]. Defects (4–12 mm) can be created in load-bearing regions, and established surgical protocols and imaging tools (e.g., MRI) support detailed analysis. Similar cartilage thickness and weight-bearing properties to humans. Minipigs are preferred for ease of handling, but larger pigs present challenges in housing and maintenance. Large joints, thick cartilage, and high loading capacities make horses suitable for late-stage studies. Ethical issues, cost, and logistical challenges limit their use to advanced preclinical testing. Anatomical location, defect size, and depth should closely mimic clinical scenarios. Immobilization or controlled rehabilitation protocols, including treadmills and swimming, may be employed. Short-term assessments include histological analysis, MRI, and arthroscopy [102]. Long-term studies focus on integration, biomechanical performance, and matrix

composition. Degradable hydrogel devices require evaluation of retention, degradation rates, and scaffold integrity over time. Endpoints should assess cellular response, tissue integration, biomechanical stability, and cartilage composition. Early evaluations may influence study continuation. Tested using histology and immunohistochemistry. Microscopic and imaging tools evaluate defect filling and scaffold integration. Load-bearing capacity and mechanical strength are assessed. Hydrogel stability and degradation products are analyzed. Toxicity, immune response, and inflammation are monitored. The clinical translation of biomaterial-based products aimed at cartilage repair constitutes a formidable challenge, often necessitating an extensive and rigorous commitment. Given that these materials are classified as high-risk medical devices, the preclinical validation of such devices through the application of predictive animal models remains a prerequisite prior to the initiation of human clinical trials [103]. This phase yields validated data concerning the safety and efficacy of biomaterial-based devices ahead of clinical evaluations. Although no specific animal model permits direct extrapolation to humans, each model can yield pertinent information that may facilitate eventual clinical translation. The selection of an appropriate animal model is critical and is contingent upon the type of device and the specific requirements for repair. The primary inherent challenges associated with preclinical validation include discrepancies in posture and loading modes between quadrupeds (serving as surrogates for human clinical investigations) and bipeds, as well as the comparatively smaller joint dimensions relative to humans, alongside the morphological diversity across species [104]. Articular cartilage plays a pivotal role, underscoring the necessity of preclinical validation. Cartilage injuries bear significant clinical implications, as the prevailing reparative strategies often exhibit suboptimal performance in restoring the tissue to its healthy and functional state. In spite of recent advancements in cartilage reconstruction, only a limited array of novel repair strategies has successfully attained regulatory approval for clinical application. Hence, an understanding of the regulatory requirements during both preclinical and clinical phases for the commercialization of such challenging products is of paramount importance. This review elucidates preclinical considerations for cartilage repair utilizing functional tissue-analogous hydrogel-based devices. While hydrogels are examined in greater detail within this discourse, it is important to note that other cartilage repair products must undergo an equivalent evaluative pathway [105]. In the application of biomaterials for cartilage repair strategies, the

principal guiding tenet is the meticulous establishment of design criteria, followed by the implementation of clinical and regulatory considerations. Moreover, the production of the device at a clinically relevant scale emerges as a critical determinant in the developmental trajectory. In the commonly utilized reparative and restorative methodologies (such as bone marrow stimulation techniques, autologous chondrocyte implantation, etc.), a primitive repair tissue does form; however, it is often deficient in mechanical strength. The fibrocartilage that is typically generated tends to degrade over time, necessitating subsequent treatment interventions [106]. Furthermore, the integration of de novo tissue with the surrounding defect is often insufficient, resulting in an incongruous surface. Nevertheless, hyaline cartilage may sporadically develop throughout the healing process. The mechanisms underlying this phenomenon remain elusive, thereby complicating efforts to consistently direct the process towards a hyaline outcome. Given the limited intrinsic healing capacity of cartilage, tissue-engineering techniques present promising therapeutic avenues [107].

Challenges and future perspective:

In the field of drug delivery, the progression of hydrogels from conventional chemical-based formulations to sophisticated supramolecular constructs signifies a fundamental transformation. This evolution has been propelled by notable progressions in material chemistry and polymer science, augmented by state-of-the-art fabrication methodologies such as three-dimensional (3D) printing and microfluidics. These sophisticated hydrogels are meticulously designed to exhibit a plethora of functional attributes, including responsiveness to targeted stimuli, the capability for direct injection into specific sites [108], and the provision of controlled drug release kinetics tailored to the unique requirements of individual patients. The ability to fabricate intricate microscale and nanoscale architectures not only enhances the versatility of hydrogels but also considerably increases their applicability in overcoming complex delivery obstacles. Challenges associated with storage, degradation, sterilization, and the delicate balance between material complexity and regulatory adherence have impeded their transition from experimental environments to clinical applications. Nonetheless, the incorporation of hydrogels within the framework of precision medicine and the burgeoning domain of biofabrication—especially in the development of bioinks for 3D bioprinting—signals the emergence of unprecedented opportunities. Such innovations, directed at the creation of personalized tissue constructs and the

refinement of drug delivery systems, underscore the necessity for design simplification to facilitate regulatory approval and commercial feasibility while preserving functional integrity [109]. RA management, hydrogel-based drug delivery systems exhibit considerable potential. RA is a chronic inflammatory condition characterized by synovial hyperplasia, cartilage degeneration, and joint destruction. Conventional therapeutic approaches frequently lead to systemic side effects owing to non-specific drug distribution. Hydrogels, with their capacity to deliver localized and sustained drug release, provide a targeted methodology that mitigates systemic toxicity while enhancing therapeutic efficacy [110]. These systems can be specifically engineered to administer anti-inflammatory agents, biologics, and disease-modifying antirheumatic drugs (DMARDs) directly to inflamed joints. Challenges particular to hydrogel-based drug delivery in RA encompass optimizing mechanical strength to endure joint movements, ensuring biocompatibility and minimal immune response, and attaining precise drug release profiles. Furthermore, the development of injectable hydrogels capable of transitioning from liquid to gel in situ presents a non-invasive delivery strategy, alleviating patient discomfort and enhancing compliance. Future prospects for hydrogel-based systems in the treatment of RA involve the integration of responsive smart hydrogels that react to environmental stimuli such as pH, temperature, and enzymatic activity, thereby facilitating site-specific drug release. Innovations in biofabrication techniques, particularly 3D printing, may further enable the production of patient-specific hydrogel implants customized to meet individual anatomical and pathological requirements [111].

CONCLUSION:

hydrogel targeting drug delivery systems present a promising strategy for the management of rheumatoid arthritis (RA), offering several advantages such as controlled drug release, enhanced bioavailability, and targeted delivery to the affected joints. These systems can improve therapeutic efficacy while minimizing side effects, addressing the complex pathophysiology of RA. Hydrogels can be engineered to respond to specific environmental triggers, such as pH or temperature, ensuring a localized release of anti-inflammatory drugs or biologics at the site of inflammation. Furthermore, the biocompatibility and versatility of hydrogels allow for the incorporation of various therapeutic agents, making them a valuable tool for RA management. While infuture research and clinical trials are needed to optimize their performance and assess long-term safety, hydrogel-based drug delivery systems have the potential to

revolutionize RA treatment, offering better disease control, improved patient outcomes, and enhanced quality of life.

ACKNOWLEDGMENTS:

The authors would like to thank the College of Pharmacy and Teerthanker Mahaveer University, for their support and encouragement.

FUNDING:

Not applicable

DATA AVAILABILITY:

All data obtained during this study are included in this manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

Not applicable.

CONSENT FOR PUBLICATION:

The work described has not been submitted elsewhere for publication, in whole or in part, and all authors participated in the work and have agreed to the content of the manuscript.

COMPETING INTERESTS:

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

ABBREVIATIONS:

RA – Rheumatoid Arthritis
ECM – Extracellular Matrix
O₂ – Oxygen
GAGs – Glycosaminoglycans
coll II – Collagen II
gG – Immunoglobulin G
ACPA – Anti-Citrullinated Protein Antibodies
HLA – Human Leukocyte Antigen
APC – Antigen-Presenting Cell
TCR – T Cell Receptor
CD – Cluster of Differentiation
NF-κB – Nuclear Factor Kappa B
Th1 – T Helper 1 Cells
TNF-α – Tumor Necrosis Factor Alpha
IL-1 – Interleukin-1
IL-6 – Interleukin-6
ROS – Reactive Oxygen Species
ACPAs – Anti-Citrullinated Protein Antibodies
VDR – Vitamin D Receptor
UVB – Ultraviolet B Radiation
16S rRNA – 16S Ribosomal RNA
NLRs – Nod-Like Receptors
TLRs – Toll-Like Receptors
CRP – C-Reactive Protein
PM – Particulate Matter
CO – Carbon Monoxide

SO₂ – Sulfur Dioxide

NO₃ – Nitrates

O₃ – Ozone

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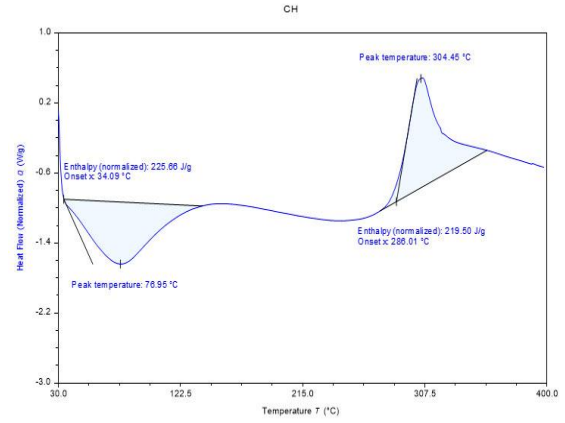
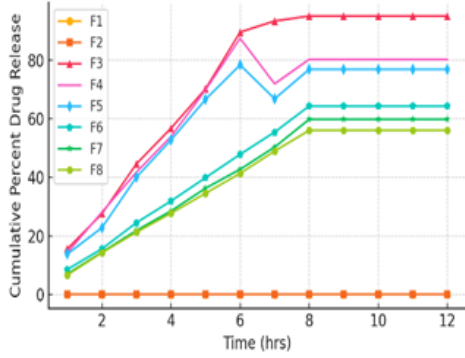
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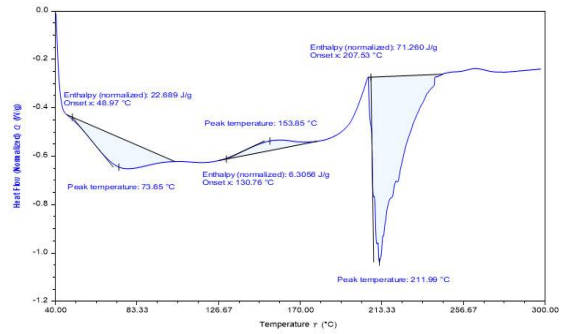
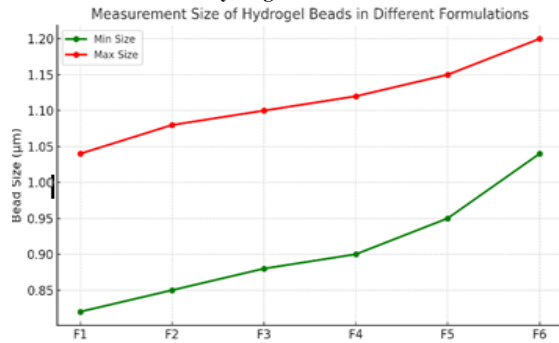
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Drug Release of Hydrogel Beads

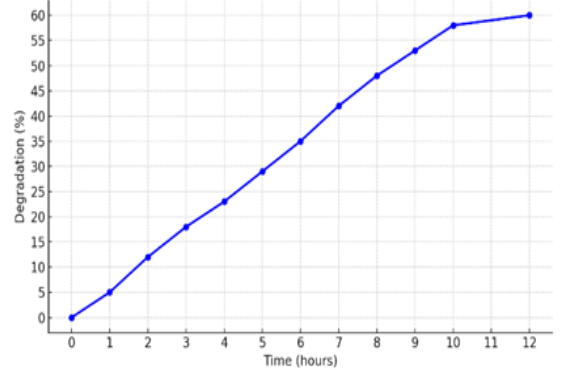
Cumulative Drug Release Profile of Hydrogel Beads (F1 to F8)



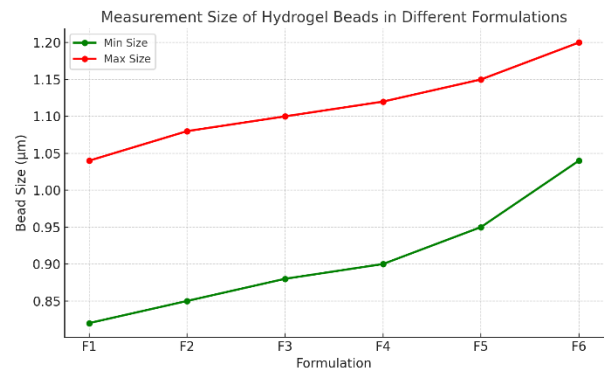
Measurement Size of Hydrogel Beads



Sr no.	Batch	%DEE±SD
1	F1	314.2± 0.14
2	F2	377.04± 0.22
3	F3	251.36± 0.27
4	F4	298.82±0.55
5	F5	397.58±0.45
6	F6	408.46± 0.12



Measurement Size of Tofacitinib



Degradation Study of Tofacitinib

DSC Thermogram

