Chapter

Smart Drug-Delivery Systems in the Treatment of Rheumatoid Arthritis: Current, Future Perspectives

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Abstract

Rheumatoid arthritis (RA) is a progressive autoimmune inflammatory disorder characterized by cellular infiltration in synovium causing joint destruction and bone erosion. The heterogeneous nature of the disease manifests in different clinical forms, hence treatment of RA still remains obscure. Treatments are limited owing to systemic toxicity by dose-escalation and lack of selectivity. To overcome these limitations, Smart drug delivery systems (SDDS) are under investigation to exploit the arthritic microenvironment either by passive targeting or active targeting to the inflamed joints *via* folate receptor, CD44, angiogenesis, integrins. This review comprehensively deliberates upon understanding the pathophysiology of RA and role of SDDSs, highlighting the emerging trends for RA nanotherapeutics.

Keywords: smart drug delivery systems, active & passive targeting, Stimuli-responsive nanoparticles, polymer-drug conjugates, Arthritic microenvironment

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune-mediated systemic, chronic inflammatory disorder characterized by progressive inflammation of joints, cell infiltration, pannus formation, synovial dysplasia resulting in cartilage destruction and bone erosion [1]. The worldwide prevalence rate of RA in adult population has been predicted between 0.5–1% and 0.92% in India [2]. Generally, prevalent in women when compared to men (3,1) at any age group. According to recent statistics given in 2019 by the Global RA network and WHO, 23 million people are affected by RA, globally [3]. RA etiology is implicated to be linked to metabolic, genetic, environmental factors, and life style of the patient [4]. While it is considered non-lethal, RA is debilitating and severely compromises the quality of life, further reducing life expectancy in patients.

Despite tremendous progress in evolving efficient pharmacological molecules for RA therapy, their efficacious delivery at the diseased joint remains a long-lasting

challenge. Over the last two decades, disease-modifying anti-rheumatic drugs (DMARDs: such as methotrexate (MTX), hydroxychloroquine (HCQ), sulfasalazine (SSZ), leflunomide (LFM), have attracted attention for effective attenuation of disease progression. Patient compliance is the primary treatment goal with glucocorticoids(GCs); e.g., prednisolone, dexamethasone, hydrocortisone, triamcinolone acetonide, and NASAID (such as ibuprofen, diclofenac, indomethacin etc) result in reducing pain and curbing disease progression [5]. Unfortunately, the associated toxicity caused by dose-escalation and long-term use with undesirable side-effects are limiting the therapeutic success. Continued medication of NSAIDs causes gastro-intestinal and renal toxicity; glucocorticoids cause hypertension, hyperglycemia, muscle wasting, osteoporosis, etc.; nausea and vomiting are common side-effects of conventional DMARDs, including gastro-intestinal irritations, headaches, insomnia, cytopenia, skin and hair damage, etc.; giving biologicals run the risk of anaphylaxis, infections, malignancy, psoriasis and other autoimmune disorders [6, 7]. Biosimilars/biologicals/Biological response modifiers like infliximab, adalimumab, rituximab etc. that have approval of Food and Drug Administration (FDA), were considered for their selective site-specific action, achieved extensive success in clinics for RA treatment. Prior reports suggest combination therapy with biologics, and synthetic DMARDs were found to be highly effective [8].

To circumvent the off-target drug induced systemic toxicity, direct drug delivery *via* the intra-articular injection to the affected joints was explored. Nevertheless, this mode of administration has several limitations, as it necessitates repeat injections in the joint, risk of infection, and joint disability. Therefore, a concerted effort for development of novel therapies are clearly warranted with a focus on targeting the inflamed joints.

Nanotherapeutics has emerged as an innovative approach enabling efficient delivery of drug for mitigating several diseases. The past decade, has seen an avalanche of publications that have increased our understanding of the pathophysiology of the affected synovial tissue in RA and equivalent progress in nanotechnology and material chemistry, generating tremendous interest in developing Smart drug delivery system (SDDS). Entrapping the anti-inflammatory drugs in SDDS strategically has potential to overcome all the barriers of normal delivery, projecting it as a promising option for site-specific delivery. Currently, RA targeting nanotherapeutics has progressed rapidly because the inflammatory microenvironments of arthritic joints mimic the tumor environment that has typical angiogenic features of neo-vessels coupled with impaired peripheral lymphatic drainage [9, 10]. This review comprehensively deliberates upon the understanding the pathophysiology of RA and role of SDDSs, highlighting the emerging trends for RA nanotherapeutics.

2. RA microenvironment

Chronic inflammation is the hallmark of RA that advances to destructive synovitis [11]. It develops in a genetically susceptible person largely due to environmental factors and related epigenetic mechanisms [12]. It predominantly indicates leukocyte infiltration, dysregulated angiogenesis, proliferation of lining layer, that alters the synovial tissue into an invasive pannus. The microvasculature of synovium is dysregulated, hence, in spite of enhanced flow of blood, the increased metabolic needs outdo the vascular blood supply, thereby creating an intense hypoxic microenvironment. However, rheumatoid factor (RF) and anticitrullinated peptide

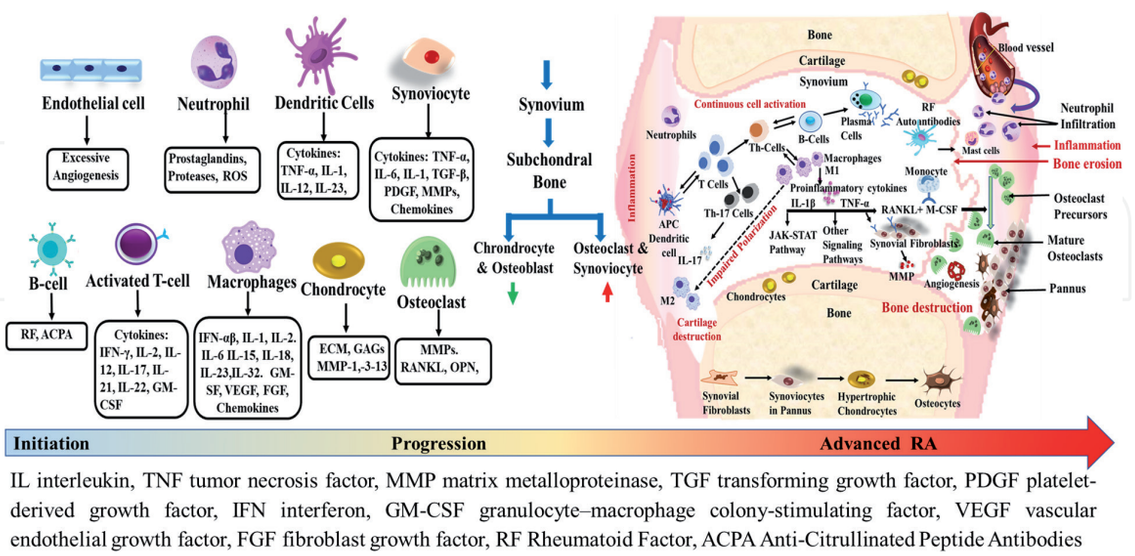
antibodies(ACPA) are induced and must exist before the onset of this disease. The heterogeneous nature of the disease manifests in different clinical forms, hence treatment of RA still remains obscure. It is well documented that synovial microenvironment has abundance of macrophages, multifaceted crosslink of immune cells secreting granulocyte colony-stimulating factor, pro-inflammatory cytokines, tumor necrosis factor(TNF-α), interleukin (IL-1), IL-6, chemokines, and degrading like MMPs that are particularly responsible for RA pathogenesis [13, 14]. **Figure 1** illustrates the network of cytokines secreted by multitude of cells involved in RA development that can be useful for assessment of disease progression along with biomarkers present on these cells.

2.1 Synovial fibroblasts (SF)

Proliferation and infiltration of SF is the key trigger for disease progression in RA. Under normal conditions fibroblasts are responsible for maintaining tissue homeostasis by modulating the inflammatory response [15]. Transcription factors like activator protein-1 and NF-κB, responsible for proliferation, activation, differentiation of fibroblasts, expression of MMPs, other matrix-degrading enzymes like cysteine proteases and aggrecanases have been observed in the synovium [5]. The genetic analysis of synovium would be useful for biological therapy as synovial tissue has a robust immune-inflammatory gene expression [16].

2.2 B cells

B cells contribute to antibody production *i.e.* RF and anticyclic citrullinated peptide antibody (ACPA), and other effector roles to entail disease progression [17]. RF and/or ACPA promotes severe bone and joint erosions as the citrullinated proteins aggravate RA progression [18]. B cells are pointedly responsible for regulation of inflammatory response by serving as antigen-presenting cells and releasing



IL interleukin, TNF tumor necrosis factor, MMP matrix metalloproteinase, TGF transforming growth factor, PDGF platelet-derived growth factor, IFN interferon, GM-CSF granulocyte–macrophage colony-stimulating factor, VEGF vascular endothelial growth factor, FGF fibroblast growth factor, RF Rheumatoid Factor, ACPA Anti-Citrullinated Peptide Antibodies

Figure 1.
Schematic illustration indicating the key events and signaling cascades involved in RA pathophysiology. Leukocytes infiltrate synovium and stimulate the inflammatory cascade, characterized by cross-talk of SF and macrophages, monocytes, mast cells, dendritic cells, as well as B and T cells. Endothelial cells facilitate angiogenesis. The advanced stage includes hyperplastic synovium, cartilage damage, bone erosion, and systemic consequence. Bone resorption by osteoclasts practically creates bone erosions. The obliteration of the subchondral bone can ultimately lead to the degeneration of the articular cartilage that is a result of reduced osteoblasts and an increase in the number of osteoclasts and synoviocytes.

pro−/anti-inflammatory cytokines that additionally control T cell differentiation and proliferation, and activation of macrophages [5].

2.3 T cells

Activated T cells comprise ≥50% of RA synovial cells, and majority are memory CD4 T cells. In terms of T cell subsets, the Th1(T helper 1) and Th17(T regulatory) are predominant, but lack Th2 subsets [5]. T cells release cytokines as their effector functions; Th1 release interferon gamma(IFN-γ), that further activate macrophages and increases its phagocytic activity. Likewise, effector cytokines of Th17 cells are IL-17A, IL-17F, IL-21, and IL-22, responsible for cell recruitment, secretion of pro-inflammatory cytokines, initiation of differentiation of B-cell, and activate NK cells [19]. IL-17 may emerge as a beneficial target for RA therapies.

2.4 Macrophages

Macrophages are tightly regulated by microenvironment signals including presence of injured cells, microbial debris, pro/anti-inflammatory cytokine or mechanical forces [19]. Depending on the cues, macrophages tend to polarize into characterized phenotypes like pro-inflammatory or immunomodulatory [20]. Taking advantage of the fundamental homing capability of macrophages to migrate to the injured/inflamed arthritic synovium, macrophages can be exploited as delivery vehicles to target specific macrophage populations to carry payloads.

2.5 Osteoclasts

Enhanced osteoclast activity triggered by disproportionate ratio of Osteoprotegerin (OPG) and receptor activator of nuclear-factor kappa-beta ligand (RANKL) are critical factors for RA progression. When RANKL is overexpressed by activated macrophages, osteoblasts and lymphocytes, it stimulates an imbalance between osteoclast multiplication and anomalous activation triggered by the binding of RANKL to RANK on the mature osteoclasts and on the cell-membranes of precursor cells of osteoclast [21]. In addition, MMP-9 and MMP-14 secreted by the osteoblasts, triggers matrix degradation in cartilage, formation of pannus, and osteoclast migration to surface of the bone. Osteoclasts significantly cause erosion of subchondral bone, articular cartilage, and the synovium.

2.6 Enzymes and other effector molecules

MMPs are the enzymes that irreversibly cause extracellular matrix (ECM) degradation, and slow-destruction of cartilage and bone in diseased joints. MMPs are zinc-dependent endopeptidases that are categorized into five sub-classes: i) gelatinases (MMP-2 and 9), ii) collagenases (MMP-1, −8 and 13), iii) stromelysins (MMP-3, −10 and − 11), iv) matrilysins (MMP-7 and -26) and v) membrane-type MMPs (MMP-14 to −17, −24 and − 25) [22, 23]. MMP-1, 2, 3, and 9 have been directly implicated in RA progression [24]. MMPs, in combination with lipases and esterases, accelerate degradation of ECM, articular cartilage and surface of the subchondral bone [25]. Ever-increasing emerging targets including these enzymes provide more options for anti-arthritic therapy with the help of targeted SDDS for RA.

3. Rationale of SDDS

Enhancement of therapeutic efficiency by 'intelligent/smart' carriers that release drugs in a controlled manner at the site of action to achieve minimal side effects are categorized as "Smart Drug delivery system" (SDDS). Maintaining optimum size and surface properties, the materials can be engineered to create nanoparticles that can maneuver the microenvironment and respond to endogenous stimuli, like increased concentration of some enzymes, redox gradient-enhanced level of glutathione, or variations in interstitial pH [26] and/ or exogenous stimuli that include temperature changes, applying magnetic field or light, and giving high energy radiation.

3.1 pH-responsive

pH, an important parameter linked to pathophysiological conditions, like inflammation can be exploited for enhanced therapeutic efficiency [27]. Reports priori give clarity that pH in normal tissue and blood is maintained around 7.4, but in arthritic microenvironment, extracellular pH values are intrinsically acidic, usually pH 6.8 [28]. The acidic pH can be attributed to the excess infiltration and activation of proinflammatory cells in the synovium, causing increased demand for oxygen and energy. Augmented consumption of glucose *via* glycolysis consequently enhances production of lactic acid, that causes local acidosis [29, 30]. Hence, the nanoparticles should be strategically designed to sensitively distinguish pH changes in inflammatory area where high disease activity and joint destruction correlates with low synovial pH. These pH-responsive nanoparticles encapsulating therapeutic molecules like NSAIDs, DMARDs etc. can be promising for RA treatment. Even at the cellular level, pH-sensitive SDDS can either stimulate drug release into lysosomes, or the late endosomes or may even trigger the escape of nanoparticles from lysosomes into the cytosol [31]. Appropriate size will enable efficient penetration in the inflamed joints, facilitated by angiogenesis during RA progression, that causes endothelial cell discontinuity leading to enhanced vascular permeability [32].

Two strategies are rationally used to design of pH-sensitive SDDS, one using materials with acid-sensitive bonds, that can be cleaved by low pH conditions allowing the release of encapsulated molecules from the nanoparticles; and secondly, using polymers (polyacids or polybases) that have ionizable groups, that undergo pH-dependent transformation and change in solubility [33]. Researchers have engineered a dual-strategy by attributing targeting abilities by surface functionalization and simultaneously using pH responsiveness to enhance therapeutic selectivity in RA.

3.2 Redox-responsive

Intracellular microenvironment can be exploited using redox responsive NPs. Reactive oxygen species (ROS) is generated primarily during oxidative phosphorylation(OXPHOS), but can further be produced by oxidative burst of activated phagocytic cells [34]. Polymers with ROS-sensitive thioketal moiety, or selenium (Se), tellurium (Te), B-based linkers in their monomeric backbone can be utilized as building blocks for the synthesis of stimuli- responsive nanoparticles. Hence, ROS can easily be monitored as an intracellular indicator [35] as chronic inflammation induces continuous production of ROS [36].

ROS concentrations in inflammatory tissues ranges 10- to 100- fold higher than normal tissues [36], thus, promising accuracy and specificity to develop the redox stimuli-responsive DDSs.

3.3 Temperature-responsive

Temperature is another crucial factor essential for release of drug [38], as the normal physiological conditions have lower temperatures compared to the inflamed RA microenvironment [39]. Therefore temperature-responsive functionalized NPs can be used to trigger the release of drug at the inflammatory site. They are designed and fabricated to retain their payloads at physiological temperature (37°C), and quick release it when the temperature is increased around 40–45°C, attributing a more efficient targeted SDDS [40]. Phase-transition behavior of the materials that are thermosensitive are used to design NPs, based on the lower critical solution temperature (LCST) of polymers/lipids whose solubility varies with changes in temperature. All excipients in a mixture are totally miscible in all amounts in LCST. In materials with transitional behavior, increased solubility is observed below LCST; and polymeric constituents are prone to swelling due to the hydrogen bonds being formed between the polymer functional groups with water molecules enabling drug loaded molecules. When temperature is raised above the LCST, a hydrophobic-hydrophilic conversion takes place, that leads to a morphological transformation from a random coil-to-globular form. Because of alterations in temperature, the hydrogen bonds breaks causing the network to collapse, and the polymer becomes insoluble, causing shrinkage in the volume and oozing-out of water molecules from inside. This transition initiates release of the entrapped payload of drugs. The application of thermo-responsive SDDS is based on the concept of exploiting the temperature difference between healthy and diseased tissues [40]. Thermal energy can be given directly, or external utilizing heat sources like NIR that may be indirectly applied in RA, that elicits a thermo-responsive behavior based on the thermo-sensitivity of nanomaterials. Typically, the requisite range of temperature fluctuates from 38–43°C [37]. The temperature-stimuli can originate from within the body, or by localized hypothermia, or hyperthermia, may provoke a response based entirely on the thermo-sensitivity of used nanomaterials. Additional advantages of thermo-sensitive NPs may be attributed to reduction in use of toxic organic solvents during fabrication, the capacity to entrap both lipophilic and hydrophilic molecules, controlled and sustained release properties. A plethora of reports using several polymers have been established for the synthesis of temperature-responsive systems, that include derivatives of poly(N-isopropylacrylamide (PNIPAAm), pluronics (poly(ethylene oxide)- poly(propylene oxide) (PEO-PPO)), poly(N vinyl caprolactam), polysaccharide spinoffs, and derivatives of phosphazene [41–43]. Researchers are making concerted efforts on achieving temperature-responsive NPs stimulated by magnetic action coupled with thermo-responsive effect by light absorption instead of temperature alone.

3.4 Light and magnetic responsive

Light-responsive systems rely on an external stimulus to activate the drug release preferably at the target site using light irradiation. NPs respond to 'on–off drug' release, as it may close/open when stimulated using light radiation. Also termed as photodynamic therapy, SDDS based on magnetic stimuli represents another external way to trigger drug release at the target site under programmable

exposure of magnetic field [37]. Iron-oxide NPs have excellent potential for smart drug delivery, as it exhibits a significant response to both light and magnetic stimuli, it can be exploited for triggering a burst release of drug at the inflamed sites of RA termed as the magneto-calorific effect and photothermal effects. Thermal properties of magnetic NPs might be conveniently modulated by modifying their own viscosity in the endo-cellular environment. Photodynamic therapy (PDT) and photothermal therapy (PTT) use photosensitizers as therapeutic molecules. Moreover, near infrared(NIR) light can efficiently infiltrate the inflamed RA joints. $Cu_{7.2}S_4$ nanoparticles triggered with NIR irradiation (808 nm, 1 W cm^{-2}) was suggested to accomplish improved bone mineral density (BMD) and bone structure and volume. It further impedes invasion to synovial tissue, erosion of cartilage and bone *in vivo*.

Huo et al. have prepared optical nanoparticles induced PTT and PDT and documented probable pathways for cell toxicity [44]. During PTT cell necrosis can be induced by NIR laser light irradiation (wavelength:1064 nm), however when given as combination therapies (PTT + PDT), evidence of both necrosis and apoptosis pathways are indicated. Furthermore, PTT-PDT combination given simultaneously, can account for immunogenic cell-death, while fluorophores can be used for optical imaging as a diagnostic tool that can be applied for RA too.

3.5 Enzyme responsive

Specific enzymes like phospholipases, proteases, or glycoside are often over-expressed in different pathological conditions, like inflammation, and can be exploited for enzyme triggered release and accumulation of drugs at the targeted site of interest [37]. Nevertheless, nature of cleavable units, the sensitivity of the delivery system can significantly influence the pharmacokinetics of entrapped payload. Further, it must be ensured that the metabolites or the degraded moieties are non-toxic and biocompatible and are cautiously eliminated from the body. Therefore, in future, enzyme-responsive nanoparticles offer tailor-made therapy according to variations in levels of disease expression. Redox- and enzyme-responsive nanoparticles are coming up as promising therapies in RA treatment.

3.6 Energy upconversion NPs

Nanomaterials with exceptional physico-chemical properties targeting the lesions can be supplemented with precise external stimuli, such as light, microwave, ultrasound, and radiation. Upconversion nanoparticles (UCNPs) synthesized from rare-earth elements that are capable of translating NIR photons that have low-energy to high-energy ultraviolet or visible photons [45]. These extraordinary NIR excitation based optical properties of UCNPs allows penetration to deep tissues with minimum auto-fluorescence background, reinforcing a wide array of diagnostic applications alongwith biomedical imaging system [46, 47]. SDDS can translate the external stimuli and equivalent energy input into beneficial effects or release the payload *via* an energy-upconversion process [48]. Ultrasound-based, photo-dynamic-based, radiation-based and microwave-based, energy-UPNPs have been widely explored in RA treatment as an alternative therapy (**Figure 2**). These developing technologies induce death of synovial fibroblasts and other inflammatory cells by generating hyperthermia, cellular ROS, mechanical and photoelectric effects [49]. Synovial cells can be directly targeted by nanoparticles to decrease bone erosion (**Table 1**) [50].

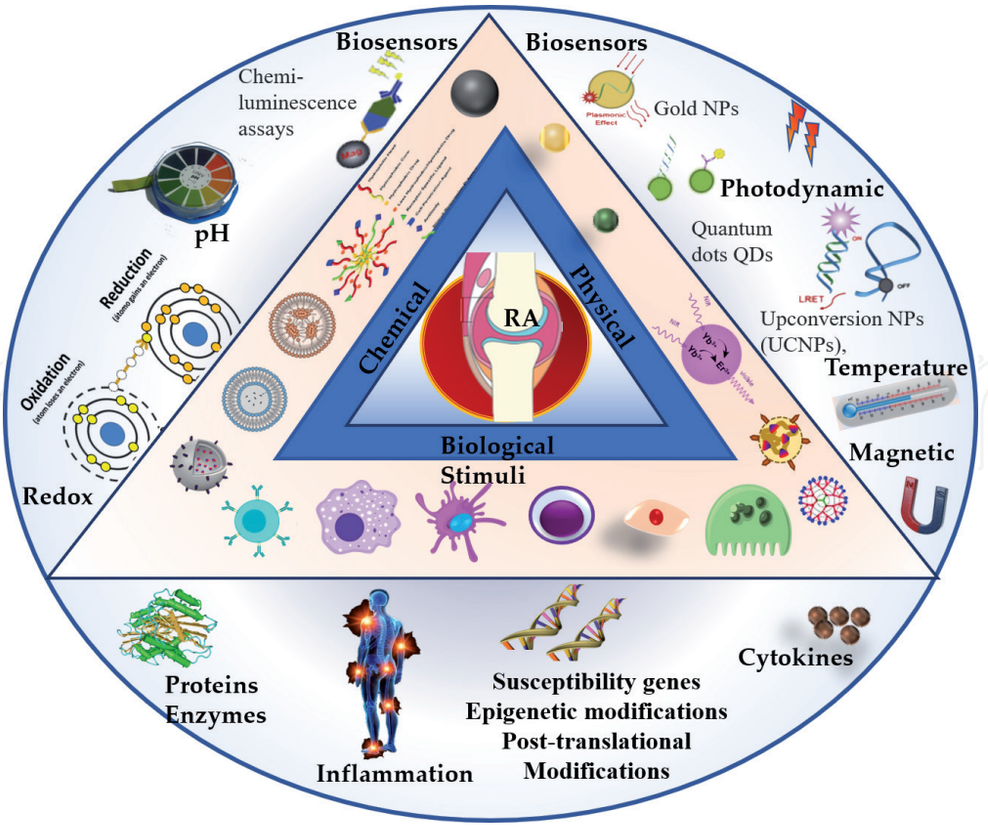


Figure 2.
Schematic representation of stimuli-responsive polymers for nanotherapeutics of rheumatoid arthritis (RA).

Stimuli category	Stimulus	Description of the system	Drug	References
Chemical	pH	PLGA-PK3-lipid-polymer hybrid nanoparticles decorated with stearic acid-octa-arginine and folic acid (Sta-R8-FA-PPLPNs/MTX) [PK3, Folate-PEG-PLGA, egg PC, and Sta-R8]	MTX	[51]
	pH	Polymeric nanoparticles surface modified with Hyaluronic acid (HA)- (HAPNPs) consisting of polyethylenimine, egg phosphatidyl-choline, and PCADK	Dex	[52]
	pH	PEGylated hyaluronic acid(P-HA) mineralized nanoparticles having a hydrophilic shell, 5β-cholanic acid as the hydrophobic core and CaP as the pH-responsive mineral	MTX	[53]
	pH	Lipid nanocarriers formed by PEG-PLGA hydrophilic shell, functionalized with folic acid (FA) ligand for targeting FA-receptor, poly (cyclohexane-1,4-diylacetone dimethylene ketal) (PCADK) & PLGA as the hydrophobic core. PCADK was used as pH-responsive material	MTX	[54]
	pH	Spherical self-assembled micelles of poly (β-amino ester)-graft-poly(ethylene glycol) (PAE-g-PEG) encapsulating MTX into the hydrophobic core.	MTX	[55]
	pH	Acetone-ketal-linked pro-drugs (AKP-dexs) pH-sensitive of dexamethasone nanoparticles	Dex	[56]

Stimuli category	Stimulus	Description of the system	Drug	References
	ROS	Folate conjugated to PEC 100 monostearate as film-forming material, and methotrexate (MTX) and catalase (CAT) co-encapsulated liposomes (FOL-MTX&CAT-L)	MTX	[57]
Physical	NIR	Gold half-shell nanoparticles functionalized with RGD to target the inflammation, encapsulating methotrexate (MTX).	MTX	[58]
	PDT/PTT	Copper (Cu)-based nanomaterials with assistance of L-cystein termed as $Cu_{7.2}S_4$ nanoparticles (NPs)	—	[59]
	Magnetic	Magnetic iron oxide nanoparticles (IONPs)	—	[60]
Multimodal Imaging	Light	Nanoparticles composed of PLGA co-encapsulating Au/Fe/Au- half-shell nanoparticles	MTX	[61]
Biological	Enzyme (MMP)	Lipid nanoparticles with PEG coating, composed of triglycerol monostearate (TGMS) and 1,2-distearoyl-*sn*-glycero-3-phospho-ethanolamine-poly(ethyleneglycol) ($DSPE\text{-}PEG_{2000}$) encapsulating Dexamethasone	Dex	[62]
	Cytokines	Nanoparticle system based on two natural polymers-N-trimethyl chitosan (TMC) and polysialic acid (PSA) encapsulated methotrexate	MTX	[63]
Combina-torial	Temp & pH	MTX loaded Gold nanoparticles and encapsulated in pegylated-poly (DL-lactic-co-glycolic acid) nanospheres	MTX	[64]
	NIR & Magnetic field	MTX-encapsulated poly(lactic-co-glycolic acid) (PLGA) (Au)/iron, (Fe)/gold (Au) half-shell nanoparticles coupled with arginine–glycine–aspartic acid (RGD)	MTX	[61]
	pH & enzyme	Micelles of polyethylene-glycol-phenyl boric acid- triglycerol-monostearate (PEG–PBA–TGMS conjugated PPT) encapsulating Dex	Dex	[65]

Table 1.
List of stimuli-responsive nanoparticles for the treatment of RA.

4. Principle of SDDS

Presently, the conventional treatments exhibit escalation in dose and systemic toxicity upon administration of drug. Most anti-inflammatory therapeutic drugs are equitoxic to both the normal cells and inflamed cells. SDDS has been well recognized in the past few decades owing to its potential for site-specific and targeted delivery [66]. Encapsulation of anti-inflammatory drugs in nanoparticles can enhance the site-specificity, reduce the dose, curtail the systemic toxicity and improve the biodistribution to targeted disease site [67]. To overcome the disadvantage of conventional delivery of drugs, selective delivery whether passive or active can be used for targeting drug to the site of action as SDDS in RA therapy.

4.1 Passive targeting

Passive targeting can be accomplished by targeting the physiological and anatomical changes in inflamed tissues, that occurred due to RA. For passive targeting, NPs do not require any surface modification, either by conjugation or by attaching a surface ligand. Various studies have shown the enhanced permeability and retention (EPR) mechanism for passive accumulation in inflamed tissues [68]. In the inflammatory RA milieu, there is evidence of angiogenesis but no evidence of displaying an abnormal lymphatic drainage [69]. The long-circulating delivery vehicles have been evidenced to specifically accumulate within the pannus of the inflamed synovium [70]. The hyperplasia in pannus exhibits a leaky vasculature due to high vascular permeability comparable to solid tumors. Consequently, taking advantage of leaky vasculature may for passive targeting is a promising option [71]. EPR allows NPs in size range from 20 to 200 nm to selectively accumulate in pannus and display on the surface of inflamed tissue. In addition to the EPR effect, hypoxic and acidic environment of inflamed joint also favors passive targeting [70]. Arthritic inflamed joint has poor oxygen delivery and increased metabolic rate due to meager perfusion into the diseased synovial joint. Therefore, the two conditions can easily be used as method of passive drug targeting in less oxygen and acidic microenvironment of RA affected inflamed tissue. NPs administrated in blood stream with hydrophobic surface are easily recognized by reticuloendothelial system (RES) such as spleen and liver, and engulfed by macrophages, consequently quickly eliminated from systemic circulation.

4.2 Active targeting

Targeted delivery involves active targeting to specific cells in the microenvironment of arthritic joints. Overexpressed receptors on particular immune cells can be targeted with its complimentary ligand that is decorated on nanoparticle surface. Several receptors are expressed by different cells, we shall be discussing a few including CD44, folate and beta-3 integrins. Targeting angiogenic vascular endothelial cells are also under investigation, with E-selectin as a promising target molecule [72]. Receptor mediated endocytosis is responsible for efficient uptake of the ligand decorated carrier molecule (ligand-receptor interaction) (**Figure 3**).

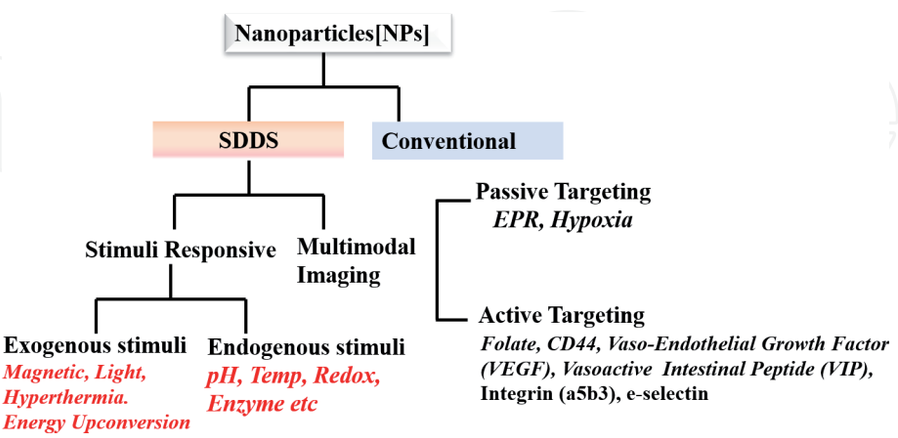


Figure 3.
Outline of the targeting strategies for nanoparticulate drug delivery systems used in RA.

4.2.1 Folic acid (FA) based active targeting

Activated macrophages overexpress folate receptor β(FR-β) in the arthritic joints [73]. Owing to post-translational modifications, the folate expressed on neutrophils

are incapable of binding to FR-β [74]. Alternatively, a functional FR-β has been identified on the activated macrophages having nanomolar affinity for folate. Hence, the FR-β receptor emerges as a useful target in various diseases including RA, osteoarthritis [75], systemic lupus erythematosus [76], atherosclerosis [77] and Crohn's disease [78]. Macrophages are key players of RA pathogenesis, as they secrete pro-inflammatory cytokines, metalloproteinases, ROS and prostaglandins. Folate is an attractive option for ease of surface modification, hydrophilicity, and stability in different solvents. Methotrexate (MTX) encapsulated folate-conjugated glycol chitosan (MFGCN) have been targeted to inflamed joint in adjuvant-induced arthritic rat model [79]. Likewise, surface of MTX-loaded liposomes was decorated with folate and were evaluated both *in vitro* and *in vivo* (DBA/1 J mice strain) [80, 81], and targeted against the FR-β on activated macrophages. Clinical efficacy of MTX in the DBA/1 J arthritic mice indicated significant expression of CD73. Since, low doses were required, this nanoformulation was economical and cost-effective. MTX loaded dendrimer nanoparticles with folic acid (FA) surface functionalization were reported to reduce progression of disease [82]. Further, authors suggested a ~ 7.5-fold increase in the maximum-tolerated dose of the MTX, when given as the formulation compared to the free MTX.

4.2.2 Hyaluronic acid (HA) based active targeting

CD44 is a glycoprotein, overexpressed on the surface of activated macrophages, present in the inflamed joints of RA. CD44 can be exploited as a prospective target in RA treatment. HA, a biocompatible natural polymer has ben explored as a ligand that effectively binds to CD44 receptor. HA coated hydroxyapatite NPs (HA-NPs) encapsulating methotrexate (MTX) and teriflunomide (TEF) - (HYA-HAMT-NP) were reported for RA treatment [83]. Results suggested that HYA-HAMT-NP could emerge as an effective delivery vehicle to circumvent hepatotoxicity caused by drugs in RA.

4.2.3 Anti-angiogenesis

Hypoxia in a critical factor in inflamed synovium that triggers neo-vascularization from existing vessels termed as angiogenesis [84]. The neo-vascularization preserves the chronic inflammatory state by engaging cells to the inflammatory site, provides nutrition and oxygen to the multiplying cells. Additionally, the enlarged surface of endothelium triggers secretion of adhesion molecules, cytokines and stimulates neutrophil infiltration as well as synovial membrane into the cartilage, causing cartilage destruction and bone erosion [85]. Promising therapies based on angiogenesis are emerging for RA therapy, where VEGF and integrins are the therapeutic targets.

4.2.3.1 Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) is an endothelial-cell-specific angiogenic factor principally secreted by SFs in the pannus. In angiogenesis, VEGF triggers multiplication and migration of endothelial cells. Further, it enhanced blood vascular permeability, stimulates maturation and maintenance of the neovessels [86]. TNF-α and IL-1, the pro-inflammatory cytokines induce the SFs and other cells to secrete VEGF, and VEGF is overexpressed at the inflamed joint owing to angiogenesis [87]. Therefore, VEGF and VEGF receptor inhibition can be an attractive strategy for RA treatment as it may effectively decrease inflammation by inhibiting angiogenesis.

4.2.3.2 Vasoactive intestinal peptide (VIP)

Vasoactive intestinal peptide (VIP) is a neuropeptide of the central and peripheral nervous system that has vasodilatory, anti-proliferative, anti-inflammatory, cell protective agent and broncho-dilatory role. The activity of VIP binds to high affinity VIP receptors, that are overexpressed on T-lymphocytes and several inflammatory cells. VIP inhibits the secretion of pro-inflammatory cytokines to act as anti-inflammatory molecule. It also promotes the secretion of anti-inflammatory molecules by stimulated innate cells [88]. Proliferating synoviocytes and activated macrophages overexpress VIP receptors in inflamed RA. Therefore, VIP receptor specific ligands can be conjugates to nanoparticles to specifically target the diseased site. Therefore, VIP can be exploited as a therapeutic agent for active targeting to RA joint.

4.2.3.3 Integrins

Integrins are the biogenic markers of endothelium undergoing angiogenesis and play a vital effector role in it. Integrin alpha-V-beta 3 (αvβ3 integrin), also referred to as vitronectin receptor are overexpressed on osteoclasts and activated macrophages of the inflamed synovium. Integrin receptor promotes angiogenesis, helps in osteoclast-mediated bone resorption, and induces pathological neo-vascularization [89]. Inhibition of αvβ3 integrin activity stimulates endothelial cell apoptosis, thereby inhibiting angiogenesis [90]. Hence, αvβ3 is considered a reliable maker for targeted delivery to RA patients.

4.2.3.4 E-selectin

E- Selectin is a glycoprotein that is associated with leukocyte rolling and adhesion and is expressed on vascular endothelium of the inflamed synovium, and promotes angiogenesis [91]. The inflammatory cytokines maintain its upregulated expression in the inflamed tissue. Therefore, expression of e-selectin can be a useful molecular target for RA therapy. Therefore, e-selectin serves as yet another attractive strategy for active targeting of the chosen delivery of drug to the diseased RA joint [92].

5. Nanotherapeutics

The assembly of stimuli-sensitive nanoparticles necessities the usage of biocompatible constituents, that can undergo supra-molecular changes in conformation, a hydrolytic cleavage, and precise protonation, etc. Polymers have maximum suitability and has been widely explored class of materials that have incredible potential. Polymers may be of natural or synthetic origin. The flexibility of the polymer sources and its ability for synthesis of various combinations of polymers has facilitated manipulation of the polymer sensitivity to specific stimuli within a narrow range [93]. Nanoparticles could be synthesized by lipid, metals and polymers. NPs decoy pro-inflammatory molecules like cytokines and ROS and sometimes osteoclast differentiation factors. Moreover, surface modification of NPs with target moiety is a extensive application in site specific drug delivery by enhancing the bioavailability of drug and reducing non-target side effects [94].

5.1 Polymer-drug conjugate (PDC)

PDC based DDS has been proposed by Ringsdrof in 1975 [95], in which a low molecular weight drug, targeting moiety and solubilizer are attached to polymeric

backbone covalently *via* bioresponsive linkers. PDC improves drug bioavailability, reduces drug toxicity, and are less toxic in nature, can easily be fabricated in regulated sizes that escape through renal filtration, exhibit increased retention time in blood circulation [96]. PDCs under investigation include N-(2-hydroxypropyl) methacrylamide, poly(vinylpyrrolidone) (PVP), hyaluronic acid (HA) and poly (ethylene glycol) (PEG) [11, 97]. N-(2-hydroxypropyl) methacrylamide (HPMA) shows improved biodegradability, biocompatibility and increased efficacy in treating RA [11, 97]. HPMA-Dex conjugates were administered intravenously into CIA induced RA model, that resulted in accumulation at the inflamed joints [98]. PEG has been used for its hydrophilicity and biocompatible properties. PEG-DEX have been developed *via* acid-labile hydrazine bond and given to arthritic rats leading to decrease in joint inflammation [99]. Recently, FDA approved two PEGylated proteins for the treatment of RA: Pegloticase (Krystexxa®) and certolizumab pegol (CIMZIA®). Although, PEG attributes stealth properties to the NPs, there are some concerns regarding PEG conjugates that include low biodegradability and the possibility to elicit an anti-PEG IgM antibody [100].

5.2 Nanoparticles

Nanoparticles are solid colloidal particles with unique physico-chemical properties such as ultra-small size, surface charge, large surface area to mass ratio Unlike polymer-drug conjugates, NPs allow encapsulation/absorption/entrapment of drug without modification. The high reactivity, diffusivity, solubility, toxicity, immunogenicity and drug release characteristics can be manipulated to make efficient delivery system. Polymeric, liposomes, micelles and metallic nanoparticles are the most commonly used nanoparticles [101].

5.2.1 Biopolymeric nanoparticles

The biodegradable backbone in biopolymeric NPs protects the drug from *in vitro* and *in vivo* degradation. Alginate, Gelatin, Pectin, Chitosan, are natural biopolymers that are highly investigated as they are biocompatible and biodegradable. Chitosan is polycationic in nature, that allows surface modification with ease and is a natural mucoadhesive. Kumar et al. reported Chitosan nanoparticles encapsulating Dexamethasone (DEX) and Methotrexate (MTX) for their *in vitro* efficacy in RAW264.7 cells and *in vivo* efficacy in arthritic rat model. Results convincingly indicated reduced toxicity and high efficacy in arthritic model [28]. The main drawback of chitosan is insoluble in alkaline and neutral medium due to absence of free amino group, but due to protonation of free amino group in acidic medium, there is enhanced solubility.

Glycol chitosan has enhanced water solubility and functional groups for further chemical modifications making it better suited as a potent drug carrier [102]. Glycol-chitosan nanoparticles(GCNPs) are biocompatible, pH responsive and biodegradable. Methotrexate (MTX) encapsulated folate-conjugated glycol chitosan (MFGCN) have been reported to target the overexpressed folate receptors β (FR-β) on activated macrophages in the inflamed joint in adjuvant-induced arthritic rat model. **Figure 4** gives a pictorial description of MFGCN that reduced the arthritic index, improved the antioxidant response and decreases pro-inflammatory cytokines and suggesting its potential in targeting activated macrophages of synovium [79].

5.2.2 Gold nanoparticles(GNPs)

GNPs can be surface functionalized through covalent bonding, by cationic polymers or physical or ionic absorption [103], functional groups like e.g. thiol, amine, and carboxyl groups that are reactive [104]. GNPs were strategically planted

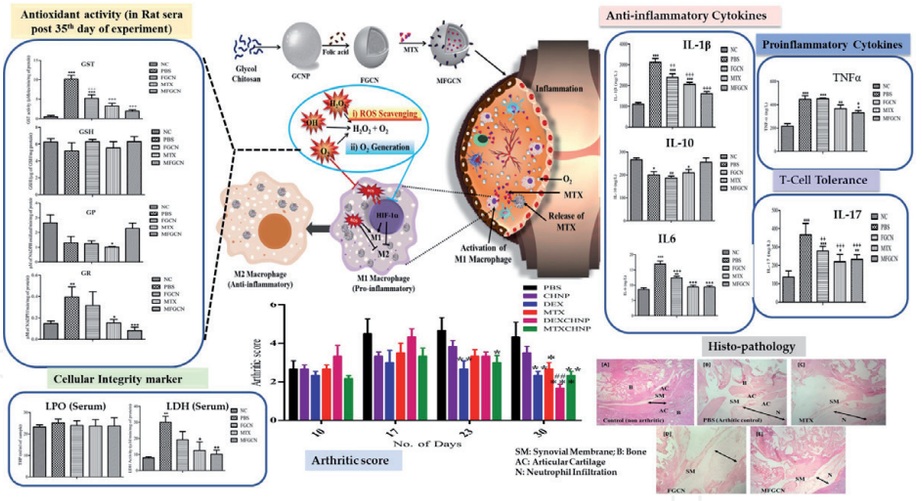


Figure 4.
*Pictorial representation of utilization of folate functionalized methotrexate loaded glycol chitosan nanoparticles (MFGCN) in treating CIA rats targeting inflammation and ROS in rat serum post 21 days of treatment (n = 6). [A] MFGCN development and active targeting of M1 macrophages in the inflammatory synovium. [B] LPO and LDH activity [C] panel showing antioxidant potential (activity quantification of GST, GSH, GP and GR) [D] quantification of TNF-α, IL-4, IL-1β, IL-10 and IL-17 [E] Representative H&E staining images of study groups comparing healthy and arthritic control groups with the treatment groups(n = 6). Data was analyzed by one-way ANOVA *p < 0.05, **p < 0.01, ***p < 0.001.*

in macrophages to target thioredoxin reductase to evaluate its antiangiogenic impact by binding to the vascular endothelial growth factor (VEGF) [105]. Lee et al. suggested MTX encapsulated RGD-attached gold half-shell NP system for RA treatment [106]. On irradiation with near-infrared (NIR), these GNPs delivered MTX to the inflamed joints, maximizing its efficacy with minimal side effects. GNPs were modified physically with Tocilizumab(TCZ) and chemically altered with an end-group thiolated hyaluronate(HA). This complex of HA-GNP-TCZ indicated a synergistic effect for its dual-functional effect on VEGF and IL-6R (receptors for IL-6) in RA treatment [107]. GNPs may block the RANKL induced osteoclast formation which leads to cartilage and bone destruction [108].

5.2.3 Liposomes

Liposomes are bilayered lipids with an aqueous core. Both hydrophobic as well as hydrophilic drugs can be encapsulated within phospholipids and the water phase cavity, making them SDDS [109]. Particle size determines the extent of accumulation at the synovium, with maximum accumulation of liposomes reported with size <100 nm diameter [110]. Therapeutic efficacy is limited due to rapid clearance from circulation *via* the RES in the liver/spleen. Surface modification by PEG enhances its hydrophilicity, makes them sterically stabilized, circumvent rapid clearance, and enhance the retention time in blood circulation [110]. Corvo et al. reported enhanced circulation and accumulation of PEG-coated SOD entrapped liposomes (mean diameter ~ 0.11 μm) at the arthritic joints [111]. Considering the dynamic microenvironment of the diseased synovium, liposomal surface can be modified with ligands, antibodies/antibody fragments or for site-specific delivery of encapsulated cargo. Recently, dexamethasone encapsulated PEG-liposome treated arthritic rats indicated accumulation of liposomes, down regulation of pro-inflammatory cytokines along with reduced inflammation of the arthritic joints [112]. Liposome tagged with a peptide sequence (CKPFDRALC-called ART-2 ligand) encapsulating DEX significantly inhibited RA progression [113].

5.2.4 Micelles

Micelles can be synthesized in small size with narrow size distribution from amphiphilic molecules that self-assemble into NPs in aqueous solution with a distinct hydrophobic cavity and an exterior hydrophilic surface. This makes them apt for intravenous injection and targeted delivery into the inflamed synovium as a consequence of extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration (ELVIS) [114]. Wang et al. reported self-assembled micelles with an amphiphilic copolymer PEG-poly-e-caprolactone (PEG-PCL), which displayed ELVIS in inflamed joints [115], but the non-biodegradable backbone of synthetic polymers caused non-specific accumulation in liver. Bader et al. [116] developed micelles from polysialic acid (PSA)-the hydrophilic polymer and synthesized micelles by altering it with N-decylamine (DA) and PCL, that formed the hydrophobic fragment. Prolonged circulation was observed with these micelles that accumulated passively at the inflamed tissue. PSA-DA micelles exhibited *in-vitro* cytotoxicity towards a synovial fibroblast cell line, the PSA-PCL micelles displayed negligible *in-vivo* cytotoxicity [117]. Further modifications were reported to improve the blood kinetic profile of the micelles. Core-cross-linked micelles were developed based on copolymer PEG-b-poly [N-(2-hydroxypropyl) methacrylamide-lactate] for targeted delivery of glucocorticoids to the RA affected joints. Here, dexamethasone was modified by methacrylated linkers *via* ester bonds, and covalently encapsulated within the polymeric structure, leading to tailorable release of dexamethasone [118]. Targeted delivery of aqueous-synthesized MTX-PEI@HA NPs to mitigate inflammatory arthritis was reported [114]. Li et al. formulated pH-sensitive micelles by conjugating hydrophobic prednisolone to PEG-derivative that confers acid-sensitive sites for attachment by forming hydrazone bonds [119]. Dual-drug loading of nimesulide and MTX in RGD-modified micelles to target αvβ3-integrin validated efficient site-specific delivery, decreased angiogenesis with minimal dose of nimesulide and MTX [120].

6. Conclusion

Multifactorial pathogenesis is the hallmark in RA causing bone fragility and functional erosion linked disability in extreme conditions. Although, conventional therapeutic formulations alone or in combination may relieve the symptoms, these are associated with complex adverse reactions. Dose-escalation, immunogenicity, systemic toxicity, and non-specific biodistribution in tissues warrant SDDS development. Stimuli-responsive NPs target specific inflammatory intermediaries, thereby suppressing the pathophysiological cascade, that may alleviate RA symptoms and delay joint destruction. Therefore, both the approaches may be exploited for achieving dose reduction coupled with drug accumulation at the targeted inflamed joint.

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