Review

Move to Nano-Arthrology: Targeted Stimuli-Responsive Nanomedicines Combat Adaptive Treatment Tolerance (ATT) of Rheumatoid Arthritis†

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Abbreviations:
RA, rheumatoid arthritis; ATT, adaptive treatment tolerance; NMs, nanomedicines; ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; NIHCE, National Institute for Health and Clinical Excellence; DMARDs, disease-modifying anti-rheumatic drugs; GCs, glucocorticoids; NSAIDs, non-steroidal anti-inflammatory drugs; MTX, methotrexate; EPR, enhanced permeability and retention; HPMA, N- (2-hydroxypropyl) methacrylamide; NIR, near infrared; LNCs, loaded nanocapsules; ROS, reactive oxygen species; CIA, collagen-induced arthritis; FDG, $^{18}$F fluorodeoxyglucose; PCL-PEG, poly (ethylene glycol)-block–poly (ε-caprolactone); AIA, antigen-induced arthritis; FRβ, folate receptor beta; PAMAM, poly (amidoamine); SRs, scavenger receptors; DS, dextran sulfate; DS-g-MTX, dextran sulfate-graft-methotrexate; HA, hyaluronic acid; LPS, lipopolysaccharide; DAPT, N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester; ML, mannosylated liposomes; HSA, human serum albumin; PEG, polyethylene glycol; VIP, vasoactive intestinal peptide; SSMs, sterically stabilized micelles; CPT, camptothecin; VECs, vascular endothelial cells; Fum-PD, fumagillin prodrug; RGD, arginine-glycine-aspartic acid; CAM, cell adhesion molecule; SA, sialic acid; VEGF, vascular endothelial growth factor; IL, interleukin; MMPs, matrix metalloproteases; NLCs, nanostructured lipid carriers; TP, triptolide; CaP, calcium phosphate; THPMA, tetrahydropyran-2-yl methacrylate; TMN, temperature-modulated noncovalent; SPL, succinylated pullulan-g-oligo(L-lactide); PLGA, poly (lactic-co-glycolic acid); MR, magnetic resonance; MPC, magnetic prednisolone microcapsules
Abstract

Rheumatoid arthritis (RA) is one of the most popular chronic autoimmune diseases characterized with persistent synovial inflammation and bone destruction. Although considerable developments have been gained in clinical treatment of RA, the major drawback to RA therapy stems from the adaptive treatment tolerance (ATT) following the long-term drug use, which causes compromised efficacy, sustained drug dose increase, and severe adverse events. To address these challenges, it is of great significance to put forward innovative therapeutic approaches for RA treatment. Nowadays, developments of nanotechnology-based nanomedicines (NMs) for RA are in progress. Multifunctional NMs with targeted stimuli-responsive features have been one of the central concepts in designing more accessible formulations for efficient RA treatment. These NMs are able to postpone RA progression effectively, because of their delivery and on-demand release of medicaments at targeted sites in response to external or internal stimuli related to the RA pathophysiology without obvious adverse side-effects on the normal tissues. Therefore, NMs have gained interest from pre-clinical research scientists as well as clinical doctors worldwide. Herein, we highlighted the recent attempts of targeted stimuli-responsive NMs for RA therapy in the last 5 years. The described progresses may pave the way to novel and highly effective RA NMs.
1. Introduction

Rheumatoid arthritis (RA) is a kind of chronic symmetric joint disease which affects 0.5%-1% of the world’s population [1a, b]. The main characteristics of the disease are autoimmunity and a series of remarkable reactions associated with inflammation in the synovium, such as sustained synovitis, progressive cartilage destruction, and bone erosion [2]. Without sufficient treatment, RA may induce accumulative joint damage and irreversible disability. Because of the highly intrinsic heterogeneity of RA, both pathogenetic mechanisms and clinical presentations vary greatly among individuals or throughout different disease stages. Despite less thorough understandings on its pathogenesis, RA is considered as a puzzled disease associated with both genetic risk factors (60%) and non-genetic risk factors (40%) [3], including genetic sensitivity, disorder of sexual hormones, microbiota infection, smoking, dust inhalation, and western diet [4]. At the initial stage, almost no symptoms or signs appear. However, with the pathological progression of RA, the expression of pro-inflammation cytokines (TNF-α, IL-1β, IL-6, and TGF-β, etc.) increases and the mucosal sites lose the initial immunotolerance [5]. As the infiltration of immune cells, autoantibodies to rheumatoid factor and citrullinated proteins expand and lead to a sharp deterioration of the joint [6]. In addition to joint swelling and the erosion of the bone and cartilage, RA can also spread to other organs and cause other complications, such as osteoporosis, atherosclerosis, peptic ulcer, liver damage and anaemia [7]. Thus, RA is a destructive disease affecting many individuals, severely reducing the quality of life and shortening the lifetime.

Considerable progress on the RA treatment has been achieved in the last decades. The American College of Rheumatology (ACR), European League Against Rheumatism (EULAR) and the UK’s National Institute for Health and Clinical Excellence (NIHCE) have given the specific guidelines on the clinical management of RA [1b, 8]. The current pharmaceutic agents for RA therapy are mainly divided into four categories, including
disease-modifying anti-rheumatic drugs (DMARDs), glucocorticoids (GCs), non-steroidal anti-inflammatory drugs (NSAIDs) and biological agents, as shown in Table 1 [9]. DMARDs are essential in interference with the inflammatory process, including conventional synthetic DMARDs like methotrexate (MTX), sulfasalazine, gold salts and leflunomide and targeted synthetic DMARDs that target Janus kinase [4]. In particular, MTX is highly effective to slow the progression of RA, and thereby is considered as the gold standard [10]. In addition to monotherapies based on only one DMARD, DMARDs are more often used in combination with other drugs to get the maximum therapeutic effects [11]. However, the long-term administration of DMARDs can cause severe adverse effects, including hepatic cirrhosis, myelosuppression, hypersensitivity and pneumonitis [12a, b, c]. As the representative agents of GCs, dexamethasone and prednisolone present fairly appealing anti-inflammatory activity and can also modify the disease in a short time. However, due to their severe side effects related to long-term administration, low-dose is recommended at the beginning of the RA treatment [13]. NSAIDs, such as aspirin and ibuprofen, are symptomatic agents that relieve swelling and pain but cannot reverse the underlying situation of the disease. They are usually used at the early stage of RA by blocking cyclooxygenases, which play a crucial role in generating prostaglandins [14]. Unfortunately, long-term usage of NSAIDs leads to increased risk of side effects including gastrointestinal bleeding, edema at inflammatory sites, and other symptoms. Recently, the emergence of biological agents has contributed great revolutions to the clinic RA therapy, such as cytokine antagonists, B cell depleting agents, T cell co-stimulation modulators and kinase inhibitors [15]. Enormous encouraging improvements have been achieved by biological agents on the treatment of patients who suffered late RA or failed by DMARDs therapy. Various antibodies have been approved for clinical usage, including adalimumab, etanercept and tocilizumab [16a, b]. However, the functions of biological agents are confined to a small portion of RA patients, and the response of RA patients who initially
have positive response to biological agents will be significantly reduced after 1-2 years treatment, resulting severe adaptive treatment tolerance to biological agents. Great concerns from clinic doctors lie on the risk of serious infections on RA patients in respect to DMARDs [11, 17]. Therefore, biological agents are still under research and need broader prospect [18].

Despite recent numerous advances in RA treatment, the major concerns on RA therapy raised from the adaptive treatment tolerance (ATT) following the long-term use of drugs. For instance, almost 50% of patients who responded to biological drug initially became tolerant to the drugs and failed to respond adequately as the development of the disease. The ATT to current drug therapy mainly lies on the poor bioavailability, high dosage requirement, and ubiquitous body distribution of the drugs upon systemic or oral drug administration, due to the encountering of a series of bio-barriers [19]. On the other hand, the intra-articular injection is suffering from poor patient compliance because of invasiveness and suboptimal efficacy arising from fast egression out from the joint [20]. Therefore, it is highly desirable to fabricate innovative drugs to combat the ATT of RA and achieve high therapy efficacy. In this regard, nanotechnology based nanomedicines (NMs) for RA is an attractive therapeutic approach in progress. As depicted in Figure 1 A, multifunctional NMs with targeted stimuli-responsive features have been one of the central concepts in designing more accessible formulations for more efficient RA treatment. These engineered NMs are able to postpone RA progression efficiently, because of their availability of medicaments at targeted sites in response to specific external or internal stimuli related to the RA pathophysiology without obvious adverse side-effects on the normal tissues. In this review, we highlight the recent attempts of targeted stimuli-responsive NMs for RA therapy in the last 5 years. The progresses described herein may pave the way to novel and highly effective RA NMs. Furthermore, the expectations of NMs in clinical therapy of RA are discussed.
2. Targeting NMs Relieve the ATT of RA

Nanotechnology refers to design and fabrication of materials and systems on the nanometers scale (1-1000 nm). With paralleling advances in nanotechnology, chemistry and biology, numerous NMs have emerged as versatile platforms for treating various human diseases ranging from cancer to inflammation, typically including liposomes, micelles, polymeric nanoparticles and metallic nanoparticles [21]. These NMs have shown advantages in solubilizing therapeutic drugs, improving penetration ability, extending retention time, and prolonging the blood circulation half-life of the cargos [22]. Therefore, NMs are able to combat the pharmacokinetic limitations associated with conventional drug formulations and show great potentials to attenuate the ATT of the existing treatments on RA. Some representative nanocarriers are presented in Figure 1B including conjugates (polymer conjugates and protein conjugates), lipid-based systems (liposomes and micelles), polymeric nanoparticles, organic nanoparticles (dendrimers and protein-based nanoparticles) and inorganic nanoparticles (metallic and carbon-based nanoparticles) with active targeting agents (Figure 1C).

2.1. NMs Overcome ATT of RA via Passive Targeting Strategies

The discovery of the enhanced permeability and retention (EPR) effect by Maeda and co-workers has been a major impetus for extensive efforts in applying NMs for chemotherapy [23]. Growing evidences have demonstrated that EPR-like effect happens in several distinct diseases, including cancer, fungal infections, various sclerosis, and rheumatology arthritis [24a, b]. It has been well documented that fibroblast-like synoviocytes, macrophage-like synoviocytes, and immune cells proliferate during the initial steps of RA [25]. As a result, the thickness of the synovium become incrassate from 2-3 cell layers to multiple cell layers, and thus induces extreme hypoxia, HIF signaling
activation, VEGF and bFGF up-regulation, and angiogenesis [26]. With the progression of inflammation, the blood-joint barrier is disrupted, resulting leaky blood vasculature system in joints. Therefore, NMs largely enable the availability of medicaments at the diseased articular sites with heightened accumulation via EPR-like effect and thus attenuate the adverse effects on normal tissues [27].

Existing studies have indicated superior therapeutic effects of NMs with appropriate sizes and surface chemical nature. It has been demonstrated that NMs bigger than 200 nm can be eliminated by spleen and NMs with a size smaller than 10 nm can pass through kidney's filtration system [28]. Moreover, larger NMs are more inclined to be phagocytosed by macrophages than the smaller ones. NMs with a size of 115 nm have proven superior anti-inflammatory performance confining the size of 45-115 nm [29]. The N- (2-hydroxypropyl) methacrylamide (HPMA) - dexamethasone conjugation based NMs were tagged with near infrared (NIR) fluorophore in the study by Quan et al. The NIR fluorescence imaging in vivo revealed the distinctive EPR-like effect mediated accumulation of the HPMA based NMs in arthritic joints. The HPMA based NMs showed much higher therapeutic index than the free dexamethasone, which was evidenced by the clinical score evaluation and micro-computed tomography [30]. The study by Boechat et al. indicated that the MTX loaded nanocapsules (LNCs) had better efficacy than the MTX solution in reducing proinflammatory cytokines and T-cell-derived cytokines such as interferon-gamma and interleukin-17A. Lower dosage was requested for MTX-LNCs to achieve comparable effective treatment. This suggests that NMs hold promising potentials to achieve effective RA treatment with lower dosages with respect to free anti-RA drugs and thus combat the ATT associated with adverse side effects [31]. Four kinds of NMs were compared head-to-head in another study, including liposomes, core-crosslinked micelles, slow releasing polymeric prodrugs and fast releasing polymeric prodrugs. All the NMs showed appealing passive targeting ability, while the slow releasing polymeric...
prodrugs showed better efficacy and longer duration than the others [32]. In a recent study, Sun and co-workers used polymeric hybrid micelles to simultaneously deliver dexamethasone and siRNA against NF-κB for arthritis therapy. The co-delivery hybrid micelles reduced the inflammation effectively by transforming the M1 macrophages to M2 state [33]. In addition to the drug conjugation and drug-loaded nanocarriers, fullerenes as the third allotrope of carbon have been exploited as innovative therapeutic NMs that can also inhibit inflammation by reducing the level of reactive oxygen species (ROS) and blunting the NF-κB signaling pathway [34]. Two kinds of fullerene derivatives have been explored for in vivo studies using collagen-induced arthritis (CIA) in DBA/1 mice and K/BxN serum transfer arthritis in C57BL/6 mice. The in vivo fluorescence imaging confirmed the inflamed joints specific accumulation of fullerene derivatives via EPR-like effect. Benefiting from the favorable targeting ability, the fullerene derivatives effectively attenuated arthritis with reduced histologic inflammation and cartilage erosion, which suggested the fullerene derivatives as promising NMs to overcome the ATT of RA and achieve efficacious therapy for arthritis [35].

The surface properties of the NMs also have great impacts on the passive targeting ability of NMs. The initial pioneering studies on the NMs used for RA treatment were based on bare (devoid of functionalization) liposomes loaded with MTX and glucocorticosteroids [36a, b]. These NMs have proven appealing specific accumulation in the RA joints via EPR-like effect. However, in absence of surface modification, the NMs were opsonized and cleaned out from the circulation in a short time. PEGylation has been used to endow the NMs with “stealth” behavior and improved targeting ability [28]. Geesa et al. monitored the targeting and therapeutic effect of PEG-liposomes loaded with prednisolone by [18]F fluorodeoxyglucose (FDG) PET/CT imaging methods. The long circulating liposomes demonstrated strong and lasting resolution of joint inflammation compared to the free drug with same dose in CIA and AIA mouse models (Figure 2) [37].
However, some concerns exist on the systematic side effects of glucocorticoid. Another glucocorticoid (dexamethasone) was delivered by poly (ethylene glycol)-block-poly (ε-caprolactone) (PCL-PEG) micelles in a low-dose to indicate the improved half-life and relative safe treatment in a rat model of arthritis [38]. The benefits of “PEGylation” were also evaluated by other nanocarriers such as polymeric nanoparticles and lipid microspheres [39].

These formations not only extend the half-life circulation and reduce renal clearance, but also enhance the solubility of the attached protein and increase the molecular weight of the protein. Until now, 11 PEGylated products have been approved for clinical use; of them, two PEGylated proteins have been developed for the treatment of arthritic conditions, including Pegloticase (Krystexxa®, Savient Pharmaceuticals, USA) and anti-TNF biologic agent certolizumab pegol (CIMZIA®, UCB, Belgium) [40a, b]. Although PEGylated NMs have shown improved bioavailability of anticancer and antiinflammation drugs over the non-PEGylated NMs, there is the so called “PEG dilemma” [41], which restricts the cellular absorption of PEGylated NMs and subsequent endosomal escape and results in lost bioactivity of the loaded drugs. A common solution to the PEGylation dilemma is to construct engineered NMs with cleavable PEG shielding [42]. Compared to the traditional PEGylated liposomes, liposomes with cleavable PEGylation showed higher cellular uptake and better bioactivity of the cargo drugs in response to the tumor microenvironment. Such an environment adaptive PEGylation strategy provides us an efficient strategy to overcome the “PEG dilemma” for NMs used for RA treatment.

Albumin, the most abundant serum protein, is considered as a beneficial attractive carrier for treating a variety of diseases. The binding properties and functions of albumin are multiple, including binding a great number of therapeutic drugs, providing colloid osmotic pressure, solubilizing agents for long chain fatty acids and providing nutrition to peripheral tissue [43]. In RA, inflamed tissues are in greater demand of energy relatively
to normal tissues, hence, albumin is no doubt a good choice to be used as carriers to load drugs [44]. As a kind of indispensable energy source, Thao et al. used tacrolimus loaded human serum albumin nanoparticles to treat the CIA mice by \textit{in vivo} administration. The albumin nanoparticles embraced dual passive targeting ability showed superior targeting efficacy. After the treatment, the symptoms of paws in CIA mice were comparable to those in normal mice. At the same time, non-target accumulation in the liver was also relatively decreased (\textbf{Figure 2}) [45].

\subsection{2.2. NMs Combat ATT of RA via Active Targeting Strategies}

In addition to the passive targeting ability by EPR-like effect, NMs are also able to achieve active targeting strategy and on-demand drug release, when combined with the unique pathophysiological signatures of the RA articular microenvironment [46]. It is an optimal approach to improve the bioavailability of RA drugs by taking advantages of NMs to target receptors or components selectively expressed at the RA sites [47]. A number of receptors have been identified as viable candidates to target macrophages (folate receptors, CD44, scavenger receptors and mannose receptors), fibroblast-like synoviocytes, and synovial microvasculature (integrin $\alpha_v\beta_3$). Numerous NMs were engineered with small molecules or natural polymers to attain active targeting functions as summarized in \textbf{Table 2}. For example, NMs showed active accumulation behaviors via efficient phagocytosis by activated macrophages, which significantly increase in inflamed joints and are characteristic players in RA. These engineered NMs exhibited more accurate delivery, relieved adverse effects and reduced inflammation than traditional formulations.

\subsubsection{2.2.1. Inflammatory Cell-Targeted NMs Overcome the ATT of RA}

In RA pathophysiology, activated macrophages are the most common cell phenotype and key effectors in the initiation and maintenance of the disease. Folate receptor beta (FR$\beta$),
scavenger receptors, CD44 and mannose receptors are all overexpressed on the surface of activated macrophages. These receptors allow internalization of modified nanocarriers loaded with therapies. Hence, targeting specific overexpressed receptors is a potential strategy to design the nanomedicine. Yang et al. synthesized folate-modified dextran-methotrexate conjugate as the prodrug which self-assembled into spherical micelles. As shown in Figure 3A, the micelles possessed improved biodistribution in lesion regions and sustained MTX release [48]. In another work, MTX was encapsulated in a liposomal formulation obtained by conjugating a hydrophobic fragment of surfactant protein to the linker and folate to improve their tolerance and efficacy. Both in vivo uptake specificity and clinical effects showed better results than non-targeted liposomes [49]. Bilthariya et al. conjugated folate to albumin nanoparticles for targeted delivery of etoricoxib. The concentration of etoricoxib significantly elevated at the inflamed sites after 24h administration indicating efficient targeting ability [50]. Folate could also be conjugated to generation 5 poly (amidoamine) (PAMAM) dendrimers loaded with MTX to treat inflammatory arthritis with comparable anti-inflammatory effects to folate-targeted MTX-conjugated polymers [51]. Aiming at the high expression of folate receptors on activated macrophages in RA, folic acid-conjugated dextran and glucose-coated iron oxide nanoparticles were developed as MRI contrast agents for diagnosis and evaluation of therapeutic effects [52].

In addition to folate receptors, scavenger receptors (SRs) are also specific biomarkers for targeting activated RAW 264.7 cells. Dextran sulfate (DS) have been selected as targeted molecule to delivery drugs in different nanoforms. Heo et al. developed an amphiphilic polysaccharide composed of 5β-cholanic acid and DS. The as-prepared DS derivative self-assembled into spherical NMs, which were selectively taken up by activated macrophages via scavenger receptor-mediated endocytosis. The DS NMs accumulated in the inflamed joints of CIA mice 12-fold higher than wild type mice and regarded as
potential NMs for RA imaging and therapy [53]. As presented in Figure 3B and Figure 3C, Yang et al. chose amphiphilic dextran sulfate-graft-methotrexate (DS-g-MTX) conjugate to form micelles directly. As confirmed by the analysis of histopathology and pro-inflammatory cytokines, DS-g-MTX showed better therapeutic effects compared to the dextran-graft-methotrexate which is also considered as a potential therapeutic option [54].

CD44 has been well documented as adhesion receptor distributed on tumor cells, activated lymphocytes and epithelial cells. CD44 is also overexpressed on the surface of leukocytes and activated macrophages in the arthritic joints under the inflammatory stimuli. Hyaluronic acid (HA) is a kind of natural polysaccharide which is widely applied in biomedical fields especially in drug delivery systems and tissue engineering for its high biodegradability and biocompatibility. HA has also been widely explored in the development of CD44-targeted NMs because of the specific high affinity between CD44 and HA. HA-MTX conjugate was prepared by ester linkage which could be cleaved in acidic environment. As expected, the acid-labile conjugate efficiently entered into lipopolysaccharide (LPS)-activated macrophages by interaction between HA and CD44. The in vivo clinical outcomes also showed great improvement compared with free MTX [55]. Afterward, a novel HA-MTX conjugate, DK226, was optimized by adjusting the linker, peptide and binding ratio of MTX. The engineered conjugate was evaluated by its pharmacokinetics properties, safety and efficacy in two distinct rat models of arthritis. The obtained results showed reduced synovial inflammation and paw thickness [56]. Previous studies have indicated that γ-secretase inhibitors are potential therapeutics for various inflammatory diseases by preventing Notch activation. Therefore, a kind of γ-secretase inhibitor (DAPT) was encapsulated in the hydrophobic core of HA nanoparticles (DNPs) which possessed both passive and active targeting ability for treating RA. As presented in Figure 4A-C, the in vivo therapeutic efficacy indicated that DNPs had lower clinical scores,
tissue damage and neutrophil infiltration in CIA mice than administering DAPT alone [57].

Mannose receptors belong to multilectin receptors that are typically overexpressed on the surface of macrophages and dendritic cells. It primarily participates in receptor-mediated endocytosis and phagocytosis. Hence, cell-specific drug delivery was achieved via this targeting approach in the study by Sultana et al. [58]. Withaferin A is a steroidal lactone that possesses a wide range of pharmacological activities, such as anti-inflammatory actions, immune regulation, anti-angiogenesis and cardioprotective effects. Mannosylated liposomes (ML) were used to encapsulate withaferin A in intent to target the synovial macrophages in adjuvant induced arthritic rats. ML-WA showed successful internalization of synovial macrophages by confocal microscopy. After treatment, the production of osteoprotegerin was upregulated and no bone erosion and cartilage degradation was found. The ameliorated severity of inflammation and bone resorption were attributed to the M1 to M2 macrophage repolarization [59]. Another drug morin which is a dietary bioflavanol was encapsulated in mannosylated liposomes (ML-Morin) synthesized by the thin film hydration method to compare the anti-inflammatory response to a reference drug (dexamethasone palmitate loaded mannosylated liposomes). ML-Morin showed better performance by significantly suppressing mRNA expression of TNF-α, IL-6 and IL-1β with increased osteoprotegerin expression [60].

Except main receptors expressed on activated macrophages, distinctive marker molecules can also be used to develop targeted drug delivery vehicle for treatment of RA. Antibodies which recognize MHC class II molecules of activated macrophages were connected to human serum albumin (HSA) NMs by using polyethylene glycol (PEG)3000 as spacer molecule. These NMs had remarkably targeting efficiency without off-target side effects [61]. Shardool et al. adopted a non-viral gene transfection strategy to repolarize macrophages in arthritic joints from M1 to M2 sub-type to treat RA for the first time.
Plasmid DNA which encodes anti-inflammatory cytokine (IL-10) was successfully encapsulated into alginate NMs that were modified with tuftsin peptide to target macrophages actively. Enhanced localization of alginate NMs were found in inflamed paws and the phenotype of macrophages was about 66% of total synovial macrophages compared to only 9% of M2 macrophage in untreated arthritic rats. Treatment tremendously reduced pro-inflammatory cytokines expression and prevented joint damage, offering a paradigm for treatment of chronic inflammatory diseases [62].

Vasoactive intestinal peptide (VIP) is a peptide hormone of 28 amino acid residues with wide biodistribution and numerous functions. It was reported that VIP takes part in regulating immune system and shows anti-inflammatory actions when bind to its receptors. VIP receptors are overexpressed on T cells and many other inflammatory cells such as synoviocytes and macrophages in RA. Consequently, some studies concentrated on VIP and its receptors for active targeting strategies. Sethi et al. constructed nano-sized sterically stabilized micelles (SSMs) modified with VIP by spontaneous interaction which protects the VIP from degradation or inactivation. By prolonging the circulation half-life of the peptide, VIP-SSM significantly reduced the severity of arthritis in animal models without side effects on the systemic functions [63]. In the research of May et al., they found a conventional anti-cancer drug camptothecin (CPT) which induced programmed cell death of cancer cells had the ability to inhibit synoviocyte proliferation, angiogenesis and matrix metalloproteinases expression. They used SSMs to load CPT and modified the CPT-SSM surface with VIP for active targeting. This study is one of the first to evaluate the in vivo efficacy of CPT against CIA with a much lower dose [64].

2.2.2. Angiogenesis-Targeted NMs Overcome the ATT of RA

Angiogenesis refers to the formation of new vasculature during either physiological or pathological processes. Likely, angiogenesis often occurs at the beginning of RA
progression. In addition to offering nutrients and oxygen to the over proliferating synovium cells, angiogenesis also enable the recruitment of inflammatory cells and improves their adhesion by $\alpha_\nu\beta_3$-integrin and E-selectin, whose species are very limited on the surface of normal vascular endothelial cells (VECs). Therefore, these adhesion molecules are considered as potential targets for active targeting strategies. There are different kinds of $\alpha_\nu\beta_3$ antagonists including RGD-mimetic cyclic peptides, linear peptides and other monoclonal antibodies [65]. An $\alpha_\nu\beta_3$-peptidomimetic antagonist was used to conjugate perfluorocarbon NMs as homing angiogenesis. Fumagillin, an antiangiogenic agent, was developed into a lipase-labile fumagillin prodrug (Fum-PD) to overcome its insolubility and instability. By delivering Fum-PD with $\alpha_\nu\beta_3$-integrin targeted perfluorocarbon NMs, the clinical score of the experimental model of RA was significantly suppressed [66].

Arginine-glycine-aspartic acid (RGD) is a kind of synthetic peptide which is extensively used as inhibitors of integrin-ligand interactions [67]. It has been explored for cancer targeting therapy for a long time and been developed for RA treatment just in a short time. Koning et al. conjugated RGD peptides to dexamethasone phosphate-loaded long circulating polyethylene glycol liposomes (RGD-PEG-L) to target $\alpha_\nu\beta_3$ integrins expressed on angiogenic endothelial cells of synovial microvasculature. In vitro results indicated that RGD-PEG-L were remarkably taken up by over proliferated human VECs and in vivo studies also proved efficacious in rat adjuvant arthritis [68].

In the process of RA, leukocytes are often recruited to synovial tissues along with a well-defined cascade of events including the capture of leucocytes from the flowing blood, adhesion to the inflamed endothelium and the migration of leucocytes [69a, b]. The selectins is a type of the cell adhesion molecule (CAM) which controls the whole cascade process. Among the whole selectin family, E-selectin is the most extensively investigated. The E-selectins are expressed on endothelial cells during RA development, thus making
them potential targets for therapeutic intervention of the disease. Sialic acid (SA), also
named as N-acetylneuraminic acid, is a monosaccharide with a nine-carbon backbone
located in the outer-most layer of cell membranes. It was reported to be an E-selection
receptor binding agent that can be used to modify the NMs. Xu et al. developed improved
multifunctional NMs, MTX encapsulated sialic acid-dextran-octadecanoic acid micelles
(SA-Dex-OA/MTX), to enhance bone repair function. The micelles had sustained drug
release behavior over 48h with reduced alanine aminotransferase, aspartate
aminotransferase, creatinine and urea nitrogen levels caused by the toxicity of MTX. In
vitro and in vivo studies showed improved accumulation in inflamed cells and arthritic
paws because of combination of SA and E-selectin receptors. Moreover, the bone mineral
density in rats treated with SA-Dex-OA/MTX was higher than those treated with Dex-
OA/MTX or free MTX. The results in Figure 4D-G indicated the bidirectional regulation of
SA in inhibiting bone erosion [70].

Vascular endothelial growth factor (VEGF) also plays a vital role in angiogenesis and
the stimulation of vascular permeability. It has been widely used as active target for
cancer therapy. Shi et al. conjugated anti-VEGFmAb to paclitaxel liposomes and showed
enhanced drug targeting ability [71]. Lee et al. employed hyaluronate (HA) -Au
nanoparticles/Tocilizumab complex to target vascular endothelial growth factor (VEGF)
and interleukin-6 (IL-6) receptor which are responsible for the pathogenesis of
rheumatoid arthritis. Furthermore, with the biocompatible and biodegradable HA on the
surface, the NMs also showed cartilage protective effects. The therapeutic effects were
confirmed by ELISA, western blot and histological analyses [72].

2.2.3 Other NMs with Potential Applications for RA Targeted Therapy
Matrix metalloproteases (MMPs) are a series of enzymes which could degrade the
components of extracellular matrix. Their physiologic expression is low in normal tissues,
whereas it considerably increases under pathologic conditions in RA [73]. MMPs can irreversibly degrade the collagen fibrils and proteoglycans in joint cartilage and bones. Therefore, MMP-responsive delivery vehicles are alternative strategies to allow selective release of drugs at the sites with aberrant proteolysis [74].

In addition to the passive targeting nature of albumin nanoparticles at inflamed sites, they can also be used as active targeting delivery systems due to a number of receptors mediated endocytosis such as gp18, gp30, SPARCT and FcRn [75a, b]. A lot of researches have reported good active targeting abilities of albumin nanoparticles in different cancer treatments [76a, b]. However, few articles have reported their applications in RA to date and it seems a good prospect to apply them in inflammatory-associated diseases in the future.

Besides systematic administration, a kind of oral agent was reported by conjugating bis-deoxycholic acid to 6-O-desulfated low molecular weight heparin (6DSHbD). After administrated orally, 6DSHbD was delivered to inflamed joint tissue preferentially and efficiently internalized by activated endothelial cells, in which heparin attaches to adhesion molecules to facilitate accessibility of the bile acid conjugate to membrane transporters. The intracellular heparin exerted anti-inflammatory roles via the inhibition of RhoA-dependent transendothelial recruitment of T cells in CIA mice. It seems a novel idea in treating chronic inflammatory arthritis [77].

Except conventional drug delivering methods, other approaches that are also aiming at increasing the concentration of the therapeutics at the diseased sites are developed. Garg et al. developed a transdermal hydrogel which combined a chemical enhancer with nanostructured lipid carriers (NLCs) for delivering methotrexate (MTX). Arthritis score and histopathological valuation validated highly decreased inflammation in RA animal models with the acceptably minimum side effects [78]. Numerous studies selected transdermal drug delivery system to treat RA in a topical way and verified the
effectiveness by arthritic score, radiological examinations and histological analysis [79a, b, c, d, e, f, g]. Triptolide (TP) is known as an effectual agent for treating RA, however it has a narrow therapeutic window because of serious toxicities. To solve this problem, a micro-needle array was used to delivery triptolide-loaded liposome hydrogel. The pharmacokinetic results indicated the plasma drug levels fit one-compartment open model and the pharmacodynamics study showed the treatment mitigated the swelling of the joints and reduced the level of inflammatory factors [80].

3. Stimuli-Responsive NMs Overcome the ATT of RA with Spatiotemporal On-demand Drug Release

By taking advantages of the inflamed articular microenvironment (acidic pH, hypoxia, energy over depletion, and overexpression of matrix metalloproteinases) [81], stimuli-responsive NMs can further ensure drug specificity and bioavailability while avoiding off-target side effects. Employment of stimuli-responsive NMs can offer extraordinary spatiotemporal control of both NMs accumulation and drug delivery at the RA sites in virtue of the ability to target the diseased tissues and respond to internal or external stimuli. Internal stimuli include over-expressed enzymes, increased cell metabolism, elevated redox potential and low pH, whereas externally applied stimuli facilitating "on-demand" NMs accumulation and/or drug release at RA sites including near-infrared (NIR) light and magnetic fields.

3.1. Internal Stimuli-Responsive NMs

Because of the abnormal metabolism in the inflammatory joints, the microenvironment of synovial fluid is acidic with pH as low as 6.0 [82a,b]. By taking advantages of this nature, some groups have exploited pH-response NMs for RA treatment [83a,b,c]. Aldayel et al. functionalized PEGylated PLGA nanoparticles by employing stearoyl-hydrazone-
polyethylene glycol 2000 which is a specific acid-responsive surface active agent. The PEGylation shielding of the PLGA NMs was cleavable upon their accumulation in inflamed sites with acid environment and thus realized efficient delivery of TNF-αsiRNA to inflamed joints [84]. Similarly, calcium phosphate (CaP) was used to mineralize the PEGylated hyaluronic acid derivative NMs. Because of the CaP as the protective barrier, the mineralized NMs showed pretty structural stability in physiological environment and rapidly released the loaded drug in acidic conditions. In addition, the demineralized NMs could be internalized in the activated macrophages via hyaladherins-mediated endocytosis. Thus, the NMs loaded with MTX were pH-dependent dissociation followed by dramatic promotion of drug release. In vivo study demonstrated a remarkably high paw-to-liver ratio after systemic administration and the CIA mice were effectively ameliorated with a relative high dose of MTX. It is highlighted the distinct advantages of combining major benefits in one formulation (Figure 5) [85]. Lee et al. adopted another pH-sensitive polymer, poly(tetrahydropyran-2-yl methacrylate) (poly(THPMA)), as the nanocarrier to encapsulate P2X7 receptor antagonist, which can inhibit inflammation through inactivating immune cells [86]. Because of the pH-dependent degradation and sustained drug release, the 3,5-dichloropyridine derivative encapsulated poly(THPMA) NMs were approved as appealing local drug delivery systems for controlled release of therapeutics in acidic inflammatory environments [87].

Besides the conventional medicine for relieving the symptoms of inflammatory arthritis, etanercept (Enbrel) is an emerging therapeutic protein against RA. Nevertheless, it is well known that protein and peptide therapeutics are usually limited for clinical application as their instability and low bioavailability in physiological conditions. Hence, a sophisticated approach was exploited to stabilize etanercept and prolong its therapeutic effect by using temperature-modulated noncovalent interaction controllable (TMN) complex. The TMN complex was formed by electrostatic interaction between negatively
charged temperature-sensitive amphiphilic polyelectrolyte (succinylated pullulan-g-oligo(L-lactide); SPL) and positively charged etanercept at 4 ℃. When the complex was in the body temperature of 37 ℃, the hydrophobic interaction will be increased and the salt and serum stability will also be enhanced. With the help of this intelligent nanocomplex, outstanding in vivo pharmacokinetic parameters and therapeutic effects were verified in CIA mice. It provides a new way for delivering protein or peptide without covalent crosslinking [88]. It is well acknowledged that the drug loading capacity and duration of drug release in diseased sites are key parameters in designing drug delivery systems. Fu et al. synthesized triblock copolymer micelles by introducing PEG_{6000} to load hydrophobic indomethacin for treatment of RA. The injectable triblock copolymer micelles were automatically turned into non-flowing gels at physiological temperatures with anti-inflammation efficacy maintained for more than 15 days [89]. Pua et al. also chose the thermo-responsive nanoscale polyion complex for treatment of local inflammation by nitroxide radicals [90].

3.2. External Stimuli-Sensitive NMs

Combination therapy has been proved as a superior method for long-term diseases such as cancer and rheumatoid arthritis to overcome resistance of the drugs. Near infrared photothermal therapy has become popular in recent years and is presented to strengthen the effectiveness of chemical therapy. In a work, the authors used pegylated-poly (DL-lactic-co-glycolic acid) nanospheres incorporating methotrexate and gold nanoparticles to realize a promising theranostic platform. The gold nanoparticles will allow the photoacoustic imaging, near infrared photothermal treatment and temperature-responsive drug release. By combining methotrexate and gold nanoparticles, the multifunctional NMs suggest a favorable reduction of inflammatory cytokines generated by monocytes and macrophages [91]. Lee et al. selected RGD peptides conjugated poly(DL-
lactic-co-glycolic acid) Au half-shell nanoparticles to load MTX. As inflamed joints are within the penetration depth of near infrared (NIR) light, the gold half-shells can deliver heat and drug to the inflamed parts simultaneously upon NIR irradiation. Because of this combination treatment, the NMs contained a much lower dosage of MTX (1/930 of MTX solution) indicated better therapeutic results in CIA mice. Moreover, these excellent NMs can be applied to other DMARDs or additional inflammatory diseases [92].

Another multifunctional NMs that were designed to fulfill targeted and combined treatment by fabricating poly (lactic-co-glycolic acid, PLGA) gold/iron/gold half-shell nanoparticles connected with arginine-glycine-aspartic acid (RGD) to deliver MTX to the inflammation region in CIA mice. These innovative NMs could be applied for in vivo multimodal imaging including NIR absorbance imaging and T2-magnetic resonance (MR) imaging. Moreover, upon the combined chemo-photothermal treatment and magnetic targeted treatment, these NMs realized prolonged retention time in diseased sites and increased therapeutic effects with only 0.05% dosage of MTX compared to free drugs (Figure 5) [93].

There are also several ferromagnetic nanoformulations used in designing targeted drug delivery systems. It was reported that a kind of magnetic prednisolone microcapsules (MPC) had great targeting ability at arthritic joints. MPC was developed via layer-by-layer assembling and the obtained results justified the adaptability of MPC for future application in magnetic targeting therapy of RA [94]. Another superparamagnetic iron oxide nanoparticles were applied to target T cells for in vivo magnetic resonance imaging in RA. Monoclonal anti-CD3 antibody was conjugated to carboxylated-PEG-SPION (IOPC) to target T cells. MRI experiments were consistent with immunohistochemical results indicating the accumulation of iron oxide nanoparticles in the corresponding regions. It may offer a prospect for treating other T cell-associated diseases [92].
4. Concluding remarks

Rheumatoid arthritis is a complicated disease that can be influenced by various factors. Although many studies are still necessary to uncover the conundrum of RA, considerable progress on the RA treatment has been achieved in the last decades. The treatment strategies for RA basically encompass DMARDs, GC, NSAIDs, and biological agents. However, the long-term use of the current medicines will ultimately lead to compromised effectiveness, sustained dose increase, and severe adverse events, which are defined as the ATT of RA to the current therapy. Despite the fact that biologic agents showed a tremendously increasing interest in recent years, the functions of biological agents are confined to a small portion of RA patients and long-term use related ATT is also inevitable. More comprehensive studies and careful evaluations are needed on biological agents before their wide use for RA treatment in clinic. The emerging NMs-based therapeutic approaches have been in progress with great potential to combat the ATT of RA. By in combination with the distinct pathophysiological features in the diseased articular microenvironment of RA, engineered NMs are able to postpone the RA progression efficiently, because of their targeting delivery and on-demand release of medicaments at diseased sites in response to some external or internal stimulus related to the RA pathophysiology without obvious adverse effects on the normal tissues. The current review is a purpose to summarize different treatment strategies guided by the features of RA in the last 5 years. Up to now, most of the studies have been focused on cancer therapy, however, less attentions have been paid to the field of RA. It is considered that the RA and cancer share some similar pathophysiological features during the occurrence and progression of the disease. Therefore, targeting strategies used in cancer would also benefit the NMs based RA therapy [95]. In addition, high interpatient variability of synovial pathophysiology is increasingly accepted as characterized by different molecular signatures, levels of inflammation and immune cell infiltrates [96]. Thus, aiming at
individual expression profiles, tailored drug delivery approaches are much prospective in
the future. Although numerous encouraging results have been achieved from many
studies, more thorough and careful studies are highly desired, especially on the
sophisticated manufacture, therapy efficacy, and long-term safety of the NMs, before their
reliable application in clinic. NMs based RA therapy approaches are emerging yet
promising and may contribute to articular diseases market. Unless there is applicability in
clinic, the innovative NMs will remain as mere theory. Nevertheless, we have confidence to
believe that engineered NMs will certainly have crucial impacts on RA treatment.

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of Sciences (No. XDA09030301).

Conflict of interest

The authors declare no financial or commercial conflict of interest.

References


Table 1. Current therapeutic agents for RA

<table>
<thead>
<tr>
<th>Category</th>
<th>DMARDs</th>
<th>GCs</th>
<th>NSAIDs</th>
<th>Biological agents</th>
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<tbody>
<tr>
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<td>Methotrexate</td>
<td>Dexamethasone</td>
<td>Aspirin</td>
<td>Adalimumab</td>
</tr>
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<td>Drugs</td>
<td>Sulfasalazine</td>
<td>Prednisolone</td>
<td>Celecoxib</td>
<td>Etanercept</td>
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<td>Leflunomide</td>
<td>Budesonide</td>
<td>Ibuprofen</td>
<td>Tocilizumab</td>
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<td>Hydroxychloroquine</td>
<td>Diclofenac</td>
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<td></td>
<td>Rituximab</td>
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<td>Anakinra</td>
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Table 2. Summary of active targeting strategies

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<tr>
<th>Target</th>
<th>Carrier</th>
<th>Therapeutic Drug</th>
<th>Nano-formulation</th>
<th>Size</th>
<th>Reference</th>
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<tr>
<td>folate receptor</td>
<td>dextran</td>
<td>methotrexate</td>
<td>micelle</td>
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<td>DOPE/CH/DSPE-MPEG albumin PAMAM</td>
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<td></td>
<td>etoricoxib</td>
<td>nanoparticle dextrimer</td>
<td>215.8±3.2nm</td>
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<td>scavenger receptor</td>
<td>Dextran sulfate</td>
<td>methotrexate</td>
<td>nanoparticle</td>
<td>200nm</td>
<td>59</td>
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<td>Dextran sulfate</td>
<td>methotrexate</td>
<td>micelle</td>
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<td>Hyaluronan</td>
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<td>nanoparticle</td>
<td>200-300nm</td>
<td>63</td>
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<td>mannose receptor</td>
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<td>DSPC/Chol/F-DHPE/Mannose</td>
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<td>liposome</td>
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<td>VIP receptor</td>
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<td>dexamethasone</td>
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<td>72</td>
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<tr>
<td></td>
<td></td>
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<td>liposome</td>
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<td>E-selectin</td>
<td>Sialic acid-dextran-octadecanoic acid</td>
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<td>117.33±5.77</td>
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</tr>
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Figure legends

Figure 1. Schematic illustration of engineering NMs used for management of rheumatoid arthritis (A) and the summary of NMs (B) and targeting agents (C).

Figure 2. Representative $^{18}$F FDG PET/CT images of antigen-induced arthritis (AIA) C57BL/6J mice at day -3 and day 4 after treated by prednisolone-containing PEG-liposomes (A-B) and empty PEG-liposomes (C-D) respectively. The therapeutic effects of two liposomes were evaluated by arthritis score and the inflammatory infiltrate and bone erosion were also evaluated by histological score (E-G) [37]. Schematic illustration of delivering tacrolimus-loaded albumin nanoparticles to rheumatoid arthritis tissue sites (H) [45].

Figure 3. Schematic presentation of dextran-methotrexate/folate conjugate in treatment of CIA mice (A) [48]. Schematic representation of dextran sulfate nanoparticles as targeted nanocarriers for RA therapy (B). Targeting ability of DSNPs in WT and CIA mice by immunohistochemistry (C) [54].

Figure 4. Schematic illustration of hyaluronan nanoparticles for inflamed tissues-targeted drug delivery (A). Paw images and mean clinical scores of CIA mice in different treatment groups (B-C) [57]. Synthetic procedures of sialic acid-dextran-octadecanoic acid conjugate (D). Evaluation of the targeting ability of SA-Dex-OA micelles in vitro (E-G) [70].

Figure 5. Schematic illustration of biomineral-installed hyaluronan nanoparticles in actively delivering drugs to the inflamed joints (A) [85]. Composition structure of the MTX-Au/Fe/Au Plasmonic NPs (B). Elucidation of magnetic targeted photothermal
treatment by NIR irradiation (C). Dual model imaging ability of MTX-Au/Fe/Au Plasmonic NPs (D) [93].

**Figure 1.**
Figure 2.

A. Long circulating Liposomes (day 0)

B. 10 mg/kg PLP-containing Long circulating Liposomes (day 0)

C. Day -3

D. Day 4

E. Arthritis score (0-4)

F. Bone erosion

G. Histological score

H. Normal Joint vs. Rheumatoid Arthritis

- Synovocyte accumulation & angiogenesis
- Inflamed synovial membrane
- Bone & cartilage erosion
- Inflammatory cytokines & Angiogenic factors
- Albumin permeation & Targeting

- HSA
- TAC

- Size = ~150 nm

- Mouse: Blood vessel
Figure 3.
Figure 4.
Figure 5.