

Nano-medicine in Treating Rheumatoid Arthritis

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Abstract. Rheumatoid arthritis (RA) is a clinically regular systemic immune disease caused by multiple genes or other factors. In a long time, scientists have taken many treasures to treat this disease. Due to the multiplicity of rheumatoid arthritis and the adverse effects of traditional drugs for the treatment of rheumatoid arthritis, scientists are actively trying to develop new technical methods, such as combining nanotechnology with traditional drugs to improve drug delivery efficiency and reduce the adverse reactions caused by traditional medicine. There are some typical pathological features in the development of rheumatoid arthritis. This review will focus on the theme of "Designing different nanomedicines based on pathological features" and divide the review into three parts: passive targeting, active targeting, stimulus-responsive targeting. In this review, the current nanomedicines for the treatment of rheumatoid arthritis are classified and summarized, with the prospect of future development of the technology at last.

Keywords: Rheumatoid arthritis, Immunology, Nanoparticle, Active targeting, Passive targeting.

1. Introduction

Rheumatoid Arthritis (RA) is a very common and typical systemic immune system disease. In developed countries, rheumatoid arthritis affects 0.5-1.0% of adults. Unfettered rheumatoid arthritis can invade many joints of the body, such as knee joints and elbow joints. The main symptoms are symmetrical swelling, morning stiffness, and continuous decline in mobility. At present, the pathogenesis of rheumatoid arthritis is not very distinct whose formation is related to a variety of factors. According to extent studies, inborn factors and smoking plays an important role in the pathogenesis of RA relatively [3-4], with affecting the subsequent immune response: B cells and T cells are activated and then produce autoantibodies (rheumatoid factor, anti-citrullinated protein antibody). The recruitment of activated T cells induces activation of macrophages and excessive production of inflammatory cytokines which include tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6. Cytokines also stimulate the proliferation of synovial fibroblasts, forming pannus that can invade cartilage and bone, leading to joint destruction. In addition, vascular endothelial growth factor (VEGF) produced by synovial fibroblasts and other cells stimulates angiogenesis, persisting the inflammation by collecting more inflammatory leukocytes.

This article reviews recent research progress in the treatment of rheumatoid arthritis with nanoparticles, including three nano-targeted drugs. Comparatively, these treatment strategies show much more potential in improving the therapeutic effects than traditional drugs.

2. Traditional treatments of rheumatoid arthritis

At present, in view of the proven pathogenesis, four drugs mainly targeted at rheumatoid arthritis have been developed: Disease-modifying antirheumatic drugs (DMARDs), Glucocorticoids (GCs), Non-steroidal anti-inflammatory drugs (NSAIDs) and biological agents.

2.1 Disease-modifying antirheumatic drugs (dmards)

There are many kinds of DMARDs. Among the DMARDs, due to the good therapeutic effect of MTX, it has now been used as one choice for the treatment of RA. At the same time, it is also one of the first-line drugs recommended by domestic and foreign treatment guidelines. MTX is a folate analog, which reduces the synthesis of tetrahydrofolate by inhibiting dihydrofolate reductase, blocks DNA synthesis in cells, inhibits the proliferation of FLS, and effectively delays bone destruction. Although MTX has superior efficacy, in clinical practice, because of its adverse reactions containing

blood cell reduction, infection, liver damage, skin mucosal toxicity and hypersensitivity pneumonia, we usually can only take low doses treatment programs. Even so, up to 30% of patients stop methotrexate within 12 months of starting treatment as a result of toxicity.

2.2 Glucocorticoids

The mechanism of action of glucocorticoids (GCs) against RA is immunosuppression, leading to a temporary and significant decrease in peripheral blood lymph and monocytes, inhibiting cell-mediated immune responses, the chemotaxis of monocytes and macrophages against the initial antigen attack, the synthesis and release of cytokines, etc. GCs have the strongest anti-inflammatory effect and are the first choice when they need to act quickly. They are often used in combination with DMARDs that improve the patient's condition. However, GCs cannot cure RA and have serious adverse reactions (atherosclerosis, osteonecrosis, etc.), which will recur after stopping the drug.

2.3 Non-steroidal anti-inflammatory drugs (nsaids)

Nonsteroidal Antiinflammatory Drugs (NSAIDs) are also commonly used drugs in the clinical treatment of RA, which can not only efficaciously relieve patients' joint swelling and pain but improve systemic symptoms.

The main mechanism of NSAIDs is to reduce the production of prostaglandins by inhibiting the activity of COX2 in progress, thereby reducing inflammation. However, these drugs cannot restrain the deterioration of RA and will have significant side effects after a long-term therapy, including central system symptoms (headaches, tinnitus, etc.), digestive system symptoms (ulcers, bleeding, perforation, etc.), cardiovascular damage (heart Failure, myocardial infarction, etc.), liver and kidney dysfunction.

2.4 Biological agents

In recent years, the development of biological agents has provided a novel treatment plan for rheumatoid arthritis, and the therapeutic effect of biological agents is splendid as well, it can reduce the progress of erosive damage, furthermore improving the quality of life of patients. The research on biological agents mainly focuses on the three directions of TNF, IL-1, and IL-6.

Among biologics, TNF is the most detailed type of biotherapeutic drug with the most detailed mechanism of action. Nowadays, five TNF- α target biologics for the treatment of rheumatoid arthritis have been approved globally, namely Infliximab, Adalimumab, Etanercept, Golimumab, and Perceli Benzumab. In view of the elevated level of TNF- α in inflammatory musculoskeletal diseases, TNF- α is thought to mediate local bone destruction in inflammatory musculoskeletal diseases, so TNF inhibitors can effectively inhibit joint tissue damage and inflammatory response in patients. Recent data show that IL-1 receptor antagonist (Anakinra), IL-6 receptor monoclonal antibody (Tocilizumab), also showed good efficacy in the early treatment of RA [14-15]. In addition, T cell and B cell targeted drugs have been put into clinical applications.

Although biological agents have achieved outstanding results, they do not have a healing effect and still have untoward effects that cannot be ignored. Blocking TNF- α -mediated signals often causes bacterial or viral infections and the development of lymphoma. Other biological agents still can increase the risk of infection.

3. Nano drugs

3.1 Advantages of nanomedicine

In summary, although traditional drugs have treated patients with rheumatoid arthritis to a certain extent, the adverse reactions caused by the non-selectivity of the drugs usually limit the increase in drug doses, while biological agents can increase the selectivity, repeated acupuncture at the joints

increases the risk of infection. So we need to explore some new drugs to improve these shortcomings in a hurry.

The emergence of nano-drugs provides a new strategy for the treatment of rheumatoid arthritis. Loading drugs on nanomaterials can directly deliver drugs to the joint cavity, avoiding high doses or frequent administration to achieve local effective drug concentrations. Not only that, but it may also increase the solubility of certain drugs and protect them in degradation in the circulation process and furtherly improves their bioavailability, reduce the off-target rate by improving drug specificity and bioavailability.

3.2 Nanoparticles for treatment

At present, for RA, the nanomedicines we use mainly include liposomes, dendrimers, micelles, nanocapsules, nanogels and many other nanoparticles. Next, we will base on the proven pathogenesis, focusing on the description of "designing different types of nanoparticles for the pathological characteristics of RA", they are classified into three strategies: passive targeting strategy, active targeting strategy, and stimulus-responsive targeting strategy.

3.2.1 Passive targeting strategy

As mentioned earlier, the site of the disease can cause vascular proliferation, so it is often used as a therapeutic target for selective drug delivery. The angiogenesis associated with inflammatory synovial tissue in RA has many similarities with the vascular remodeling found in solid tumors. The pannus tissue of RA synovial membrane is considered to show characteristics similar to the tumor site, so it will produce tumor-like enhanced permeability and retention (EPR). Through the EPR effect, nanocarriers of appropriate size will leak into the synovial tissue through the gap between endothelial cells, and stay there to slow down the release of the drug and be in RA. Due to the inflammatory response, in addition to the similar EPR effect, the recruited inflammatory cells will also absorb the drug. This phenomenon is called "ELVIS". Based on the ELVIS phenomenon of RA, passive targeting of nanoparticles can be achieved. Therefore, people have designed a variety of nanoparticles to treat inflammation.

3.2.1.1. Liposome

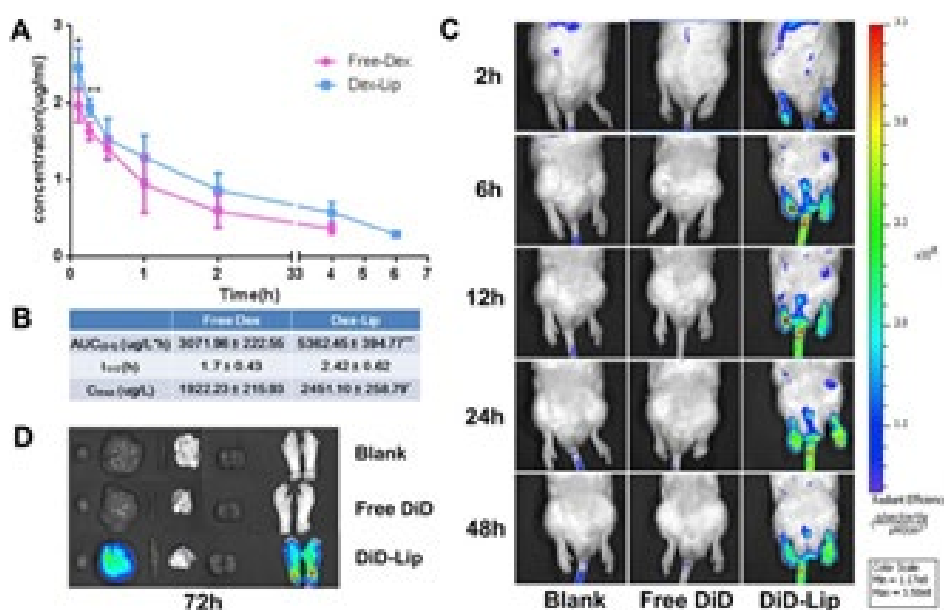


Fig. 1 The in vivo biodistribution study of DiD-Lips in arthritic rats and the pharmacokinetic study of Dex-Lips in normal rats (A) The free Dex and Dex-Lips in normal rats (n = 6) Pharmacokinetic characteristics and several pharmacokinetic parameters (C) fluorescence intensity after intravenous administration of different DiD preparations at different time points. (D) 72 hours after administration, the fluorescence intensity of rat heart, liver, spleen, lung, kidney and limbs. Rats without any treatment were used as blanks.

Liposomes have been extensively used in clinical practices, and they have proven their versatility in a variety of pharmaceutical preparations. For many years, liposomes have proven to be well-tolerated carriers, because most liposomes are composed of (semi-)natural, biodegradable lipids. At present, a number of traditional drugs are encapsulated in nanoparticles. Compared with simple free drugs, the drugs can not only selectively reach the effective site, but also take longer to act. For example, in the study of Jia et al., a liposome-based delivery system for inflammation was developed to deliver anti-arthritis hydrophobic dexamethasone. They used FDA-approved excipients for intravenous injection, and prepared Dexamethasone-loaded liposomes (Dex-Lip) by the film hydration method. The following figure shows that the Dex concentration of the drug released at 0.1 h ($P < 0.05$) and 0.25 h ($P < 0.01$) The time point was significantly higher than that of the free Dex group. Compared with the free drug solution group, the Dex-Lips group showed significantly higher area under the curve (AUC $0 \rightarrow t$) ($P < 0.001$) and C max ($P < 0.05$). The half-life of Dex in the Dex-Lips group was also slightly higher than that in the free Dex group. And the Dex-Lips group has better targeting ability.

Except the traditional medicines, the next generation drug biologics are also matched with liposomes to achieve better results. Superoxide dismutase (SOD), an enzyme with anti-inflammatory activity that catalyzes the conversion of superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). The enzyme was encapsulated in liposomes for the first time in 1985, reducing toxicity in RA and obtaining higher therapeutic effects. Later, SOD was loaded in different liposome formulations, and it was found that small PEG-liposomes can more effectively deliver SOD to the arthritic site, thereby reducing paw edema more effectively. In 2015, the latter research made new progress. The covalent attachment of SOD to the surface of long-circulating liposomes shows higher anti-inflammatory activity than liposomes encapsulating SOD, which indicates that the position of the drug in the nano-carrier may also have a major impact on therapy.

3.2.1.2. Polymer

Polymer nanoparticles are also widely used in nanomaterials. Unlike liposomes, polymer nanoparticles not only have good biodegradability and biocompatibility, but also these nanoparticles show considerable potential for modification, pharmacokinetics control release ability, and can carry various therapeutic agents. It has been reported that Japanese scientists have found DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate (BSP) release sustained drug release in vitro for more than three weeks. In the rabbit model of antigen (ovalbumin)-induced arthritis, the joint swelling was significantly reduced by injecting BSP-loaded nanoparticles within 21 days after joint inflammation. During this period, serum antibodies against ovalbumin continue to decrease, and the uptake of synovial cells can prevent cartilage degradation.

With the availability of transporting traditional drugs, in recent years, the utilization of polymer nanomaterials to carry small RNA has also been exploited. As we all know, in the pathogenesis of RA, pro-inflammatory factors play an important role. Among these cytokines, $TNF-\alpha$ secreted by activated macrophages occupies the main position, which induces the release of other cytokines in RA synovium, bone resorption and chronic inflammation. Many biological agents appear in succession for this target. However, considering the side effects of biological agents, scientists study a new treasure called RNA interference (RNAi), and it is able to silence the gene expression for treating large number of diseases, including viral infections, cancer and autoimmune diseases. Although siRNAs are powerful reagents, the process of delivering them to target cells is also challenging because of their poor stability in body fluids. Nano-drug delivery systems can be applied to siRNA to improve its poor water solubility and low bioavailability, and it has the capacity to load a sufficient number of therapeutic drugs, extend the circulation time and deliver the drugs to the target area at the same time. In a specific case, Lee et al. prepared siRNA/thiol glycol chitosan nanoparticles for the treatment of RA and found it in a CIA-induced mouse model that the psi-tGC-NP system is enhanced the aggregation of poly-siRNA (see figure below) in arthritis joints. After the free siRNA released from psi-tGC- NPs is endocytosed by activated macrophages, the target mRNA can be

effectively knocked out. Moreover, blocking TNF- α by psi-tGC-NPs can inhibit paw swelling, which is equivalent to the efficacy of the anti-rheumatic drug MTX.

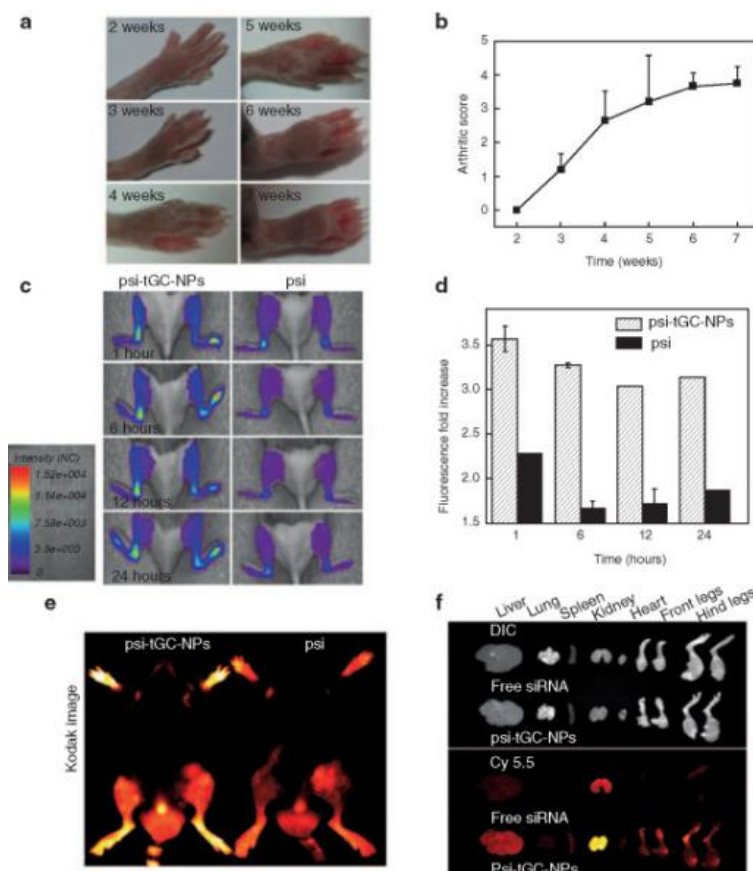


Fig. 2 In vivo imaging of psi-tGC-NP accumulated in the arthritic joints of collagen-induced arthritis (CIA) mice. (A) Macroscopic paw swelling in the CIA model (b) Arthritis score in the CIA model. (C) After intravenous injection of psi-tGC-NPs or free poly-siRNA, in-vivo near-infrared fluorescence (NIRF) tomographic images of arthritic joints. (D) The fluorescence intensity of FPR-675-labeled poly-siRNA at each time point was recorded on the hind limbs (N = 3). (E) NIRF reflection image of CIA mice 24 hours after intravenous injection of PSI-TGC-NP or free poly-siRNA (f) Isolated NIRF image of dissected organs 24 hours later.

3.2.1.3. Other nanoparticles

Liposomes and polymers are mostly used nanomaterials. In addition, there are other nanoparticles that are also applied in the treatment of RA. Metal nanoparticles have excellent properties and can be modified into multiple functional groups. The manganese ferrite/cerium dioxide co-decorated mesoporous silica nanoparticles (MFC-MSNs) synthesized by Kim et al. are effective in removing active oxygen (ROS) and the production of O₂, which can reduce M1 macrophages and induce polarization of M2 macrophages, reduce hypoxia, inflammation and other pathological features of the joints. Silica particles are frequently used in inorganic non-metallic particles. Li et al. injected modified mesoporous silica nanoparticles (MSN-CC-PEI) with type 2 hyaluronic acid synthase (HAS2) into the joint cavity.

3.2.2 Active targeting strategy

After finishing passive targeting, the inflammatory environment of RA can be used to design an active targeted drug delivery system. Active targeting is designed for the transformation of cells involved in the pathogenesis and development of RA, such as the up-regulation of specific surface receptors or the conversion of phenotype. In consideration of these surface features, we can modify the corresponding ligands on the nanoparticles to further enhance the ability of nano-targeting. Next, I will classify active targeting agents into the following categories according to the types of nano

surface modifiers: small molecule modified nanoparticles, polymer modified nanoparticles, peptide modified nanoparticles, protein modified nanoparticles, and bionics (Cell membrane) nanoparticles.

3.2.2.1 Targeted delivery mediated by small molecule modification

Folic acid (FA) and its receptors (FRs) are highly expressed in tumor cells and activated macrophages of RA patients [37-38]. According to reports, compared with normal rats, the FRs of AIA rat synovial macrophages have a higher affinity for FA. Based on the former studies, folic acid is often thought as a strategy for active targeting. In a research, a folic acid-modified dextran-methotrexate conjugate (called Dex-g-MTX/FA) was synthesized with an untargeted dextran-methotrexate prodrug (called Dex- g -MTX) as a control. After the two prodrugs self-assemble into spherical micelles, Dex-g-MTX/FA exhibits more folate receptor-mediated cellular uptake compared with Dex-g-MTX, and it has higher cytotoxicity to lipopolysaccharide-induced activation of macrophages. In addition, Dex-g-MTX/FA has better biodistribution in the inflamed area.

Sialic acid (SA) is a natural monosaccharide, mainly located on the surface of cell membranes. As a binding ligand for the E-selectin receptor, SA has been used to improve the efficiency of targeted delivery in recent studies. The sialic acid modified liposomes loaded with dexamethasone palmitate (DP), vitro and vivo experiments show that SA modified liposomes can enhance the accumulation of DP in peripheral blood neutrophils (PBN), and inhibit RA. In other words, SA-modified liposomes (DP-SAL) show a greater degree of accumulation in joints and have a stronger anti-inflammatory effect.

Hyaluronic acid (HA) is a frequently used polymer that can bind to CD44, which is found in synovial lymphocytes of RA inflamed joints. Using the combination of HA and CD44, a variety of actively targeted drugs can be designed. Studies have found that the glucocorticoid prednisolone (PD) is encapsulated in solid lipid nanoparticles (SLN) coated with hyaluronic acid (HA) to produce HA-SLNs/PD. After injecting into mice with collagen-induced arthritis (CIA), HA-SLNs/PD has lower arthritis scores after long-term use than free drugs or drugs encapsulated in non-HA SLNs, and bone and cartilage is better protected.

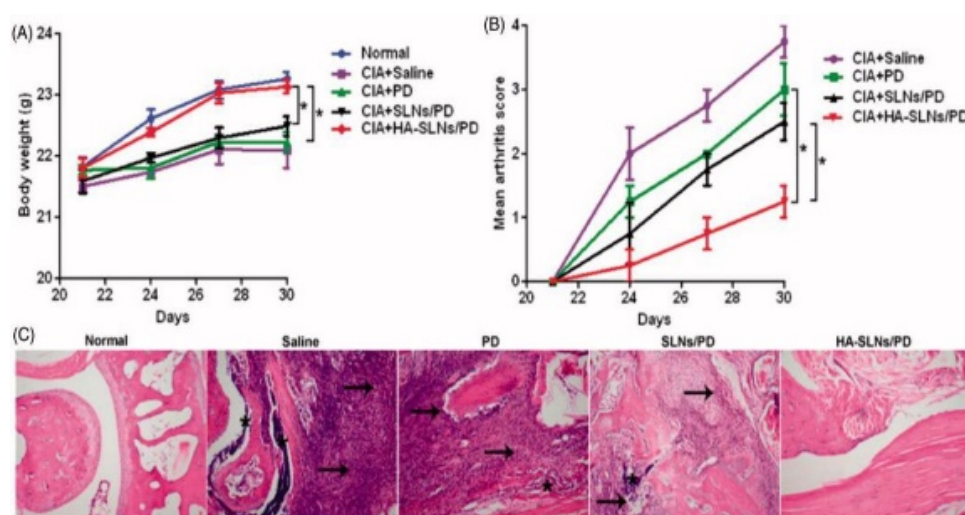


Fig. 3 The therapeutic efficacy of HA-SLNs / PD in vivo (A) Body weight of different treatment groups. The data are the mean $\pm$ SD (n = 5) (B) After different treatments, calculate the average arthritis score of the right hind limb joint. The data shown is the mean $\pm$ SD (n = 5) (C)

Photomicrographs of animal ankle joint tissue sections after different treatments. The arrow indicates the formation of finger-like pannus; the asterisk indicates the destruction of bone. Rod shape, 100 nm.

3.2.2.3 Peptide-mediated targeted delivery

Arginine-glycine-aspartic acid (RGD) and its derivatives have been broadly used to enhance the delivery efficiency of therapeutic agents. As we all know, during the development of rheumatoid arthritis, new blood vessels will proliferate. The RGD peptide shows high affinity and selectivity for

integrin $\alpha_v\beta_3$, which is the main reason of vascular proliferation. Therefore, a recent study also focused on this feature. Wang et al. found that the combination therapy of methotrexate and nimesulide mediated by RGD-modified polymer micelles showed exciting result: RGD-modified polymer micelles last longer in the circulation and have the most significant inhibitory effect on angiogenesis.

In addition to RGD, synovial fibroblast targeting peptides will also be used as a strategy. HAP-1 peptide (SFHQFARATLAS) is a synovial fibroblast-specific protein transduction domain. Injection of HAP-1 peptide into the joint will fuse with mitochondrial destruction peptide (KLAK) 2 and cause obvious apoptosis of synovial cells and reduce inflammation and synovitis. In practical examples, Vanniasinghe et al. developed HAP-1 modified liposomes, which encapsulate anti-arthritis drugs and can inhibit the development of arthritis by targeting FLS. The experimental results show that the targeted liposomes specifically bind to rabbit FLS and human FLS respectively, and compared with unaffected joints, the localization in the affected joints increases 7-10 times. What's more, prednisone and immunosuppressive peptide CP in tissue sections of rats treated with the new drug had the weakest inflammatory response. There are also HAP-1-mediated targeted liposomes loaded with NF- κ B blocking peptides, which can effectively inhibit the activity of NF- κ B and reduce zymosan-induced synovial inflammation.

3.2.2.4 Protein-mediated targeted delivery

In the treatment of RA, protein-targeted delivery mainly concentrates on the application of antibodies. The antibody will specifically bind to the antigen on the surface of the cell membrane, thereby precisely targeting the therapeutic drug to the cell. CD134 can be used as a candidate target, reducing the activated CD4⁺ cells in the body which is beneficial to the treatment of rheumatoid arthritis, while CD134 is only expressed on activated self-aggressive CD4⁺ cells. Therefore, researchers explored and injected subcutaneously anti-CD134 liposomes mixed with the cytostatic 5'-fluorodeoxyuridine into the hind paws of pre-arthritis rats, and found that these liposomes can be specific in vitro inhibit the proliferation of activated CD134 + T cells and improve adjuvant arthritis. Similarly, CD163 is not only highly expressed in tissue macrophages in the liver, spleen, and bone marrow, but also in macrophages in inflammation sites (such as atherosclerotic lesions and joint inflammation in rheumatoid arthritis). Based on this, the researchers made a hydrophobic link between the CD163-binding monoclonal antibody and polyethylene glycol-coated liposomes ("hidden liposomes"), developed liposomes that specifically target CD163. Observation of CD163-targeted liposome cells by confocal microscopy and flow cytometry revealed that the uptake rate of CD163-targeted liposomes in macrophages increased.

Albumin also has the potential for targeted delivery in inflammatory diseases. In the inflammatory joint cavity of active RA, SPARC (acid secreted protein, rich in cysteine) overexpresses in the synovial fluid and synovium of RA patients and the collagen-induced arthritis (CIA) mice, using the high affinity of albumin and SPARC, combined with MTX to make albumin-mediated nanoparticles, can effectively improve the accumulation of MTX and its safety.

3.2.2.5 Cell-membrane targeted delivery

By now, biomimetic nanoparticle drug delivery systems coated with cell membranes have received more and more attention to achieve natural targeting of diseased sites. After the germination of RA, a large number of inflammation-related cells are recruited to the inflammation site, they adhere to the inflamed endothelium, and then penetrate into the affected synovium. The use of nanoparticles coated with inflammatory cell membranes can achieve active targeting, and they also have the ability to avoid being cleared by the reticuloendothelial system, increasing their retention in the systemic circulation.

Taking into account the advantages of cell membranes, coating preparations like macrophage membranes, neutrophil membranes, platelet membranes, red blood cell membranes, etc. have been prepared. The current researches mainly pay attention to the two parts of macrophage membranes and neutrophils. Lee et al. developed macrophage-derived microcapsules (MMV) coated nanoparticles (MNP) to target RA. The study used cytochalasin B (CB) to reduce the interaction between the

cytoskeleton and cell membrane of macrophages, so that stimulate the secretion of MMV. Because MMV membrane proteins are similar to macrophages, MMV can show analogous inflammation targeting capabilities to macrophages. MMV is coated on the surface of nanoparticles to prepare MNP. MNP can be adhered to the site of RA through ICAM-1 or P-selectin, as shown in the figure below, and compared with free NP and erythrocyte membrane-encapsulated nanoparticles (RNP), MNP displayed dramatically enhanced effect in collagen-induced arthritis (CIA) mouse models, and inhibited the RA process in mice. The study predicts that MMV has the potential to become a new bionic carrier for the treatment of arthritis.

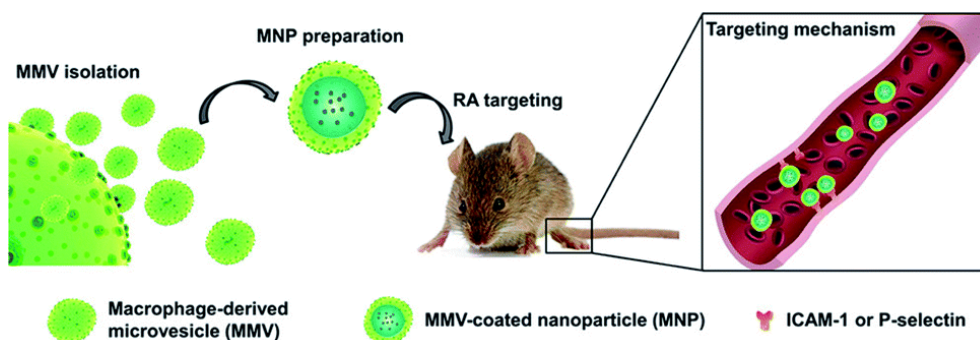


Fig. 4 Schematic diagram of the targeting site of RA's MMV coated nanoparticles (MNP).

MNP can adhere to the site of RA through ICAM-1 or P-selectin.

Zhang et al. explored a kind of neutrophil membrane-coated nanoparticles to reduce joint damage. In CIA mice and human arthritis transgenic mouse models, these nanoparticles can reduce the production of pro-inflammatory factors, inhibit synovial inflammation, protect cartilage, and show significant therapeutic effects.

3.2.3 Stimulus responsive targeting

Although passive and active targeting strategies can deliver drugs to effective sites, the release of drugs is uncontrollable. Taking into account this shortcoming, a drug delivery system based on the physical and chemical microenvironment change response is designed, which can not only achieve fixed-point release of drugs, but also achieve timed release of drugs, so as to achieve a long-term effective therapeutic effect.

In the development of active RA, a large number of inflammatory cell infiltration and invasive proliferation are accompanied by a rapid increase in cell metabolism in joint tissues, leading to the accumulation of acidosis and oxidation intermediates. In addition, inflammatory cells will produce excessive amounts of matrix metalloproteinases (MMP) [57-59] in the lesion site.

According to changes in the RA microenvironment, many stimulus-responsive nanomedicines can be designed, which will be used for diagnosis or treatment. In light of the corresponding type of stimulus, it is divided into acid-sensitive, ROS-sensitive, MMP-sensitive and other sensitive nanoparticles. The first three items are numerous in the latest researches.

3.2.3.1 Acid-sensitive type

In RA, the pH of inflamed joints is usually between 5.0 and 6.0. According to the slightly acidic microenvironment, acid sensitive substances is applied to mediate the targeting effect.

For example, Alam et al. prepared pH-responsive biomineralized nanoparticles assembled through ionic interactions. The nanoparticles consist of PEGylated hyaluronic acid (P-HA) as a hydrophilic shell, 5 β -cholic acid is as a hydrophobic core and calcium phosphate (CaP) is as a pH-sensitive mineral. Among them, CaP acts as a diffusion barrier and mineralized HANPs show pH responsiveness of MTX release kinetics after pH transformation. As a result, the nanoparticles distributed well in the arthritic paws and released MTX in response. In a recent study, a pH-sensitive TP loaded nano-drug (TP@NPs) was designed by encapsulating TP into the star-shaped amphiphilic block copolymer POSS-PCL-b-PDMAEMA. Compared with free TP, RAW264.7 cells treated with TP@NPs in vitro showed less cytotoxicity and apoptosis. In addition, the results of indocyanine green

labeling in vivo show that the compound not only has effective pharmacokinetic and pharmacodynamic characteristics, but also has obvious chondroprotective and anti-inflammatory effects. Compared with free TP, even at 50°C or higher temperature, it also has superior efficacy and negligible systemic toxicity under higher conditions.

Both of the above are acid-triggered drug releases. There is another example that the acidic environment of RA-inflamed joints may induce the shape transition of nanocarriers from vesicles to fibers, so that their retention time is prolonged.

3.2.3.2 Ros-sensitive type

The inflammatory signal cascade and tissue damage often cause the accumulation of oxidation products and oxidative stress response. The level of reactive oxygen species (ROS) in inflamed tissues is higher than that in normal tissues, so ROS sensitive groups: such as chalcogenides (sulfur, selenium), phenylboronic acid/ester (PBA/PBE), cerium dioxide (CeO₂) [64] and so on. They are considered as potential means to control their release. Fan et al. synthesized polymer micelles of selenocysteamine as ROS-responsive carrier nanoplateforms containing berberine. The therapeutic effect is accomplished by targeting mitochondria, inhibiting adipogenesis and ultimately inhibiting cell proliferation. It significantly increases the uptake of berberine in RA fibroblasts, and its efficacy in vitro and in vivo is increased tenfold. Recently, Xu and colleagues established FA-modified ROS-responsive nanoparticles by incorporating 4-phenylboronic acid (4-PBA). Nanoparticles show that H₂ generated by ROS in response to the drug release curve can effectively eliminate O₂ in activated macrophages. After loading Dex, treatment with ROS-responsive nanoparticles can significantly reduce histological damage and reduce inflammation in CIA mice.

3.2.3.3 Mmp sensitive

Disease-related enzymes are also used as specific stimuli for designing bio-responsive nanomedicines. The up-regulation of MMPs expression in inflammatory joints is a regular feature of RA. Therefore, stimulus-responsive nanocarriers based on abnormal secretion of MMPs have been extensively explored for use in controlled drug delivery in RA. Recently, He et al. reported a triglyceride monostearate (TGMS) and 1,2-distearoyl-sn-glycerol-3-phosphate-ethanolamine-polyethylene glycol (DSPE-PEG2000) co-assembled MMP responding PEGylated lipid nanoparticles, in which the ester bond of TGMS can be cleaved by MMP, and the PEGylated drug delivery vehicle can avoid the recognition and clearance of the reticuloendothelial system (RES). The circulation time of lipid nanoparticles in the blood is prolonged and the targeting effect is good. After intravenous injection into arthritic rats, Dex-encapsulated MMP-reactive PEGylated lipid nanoparticles can reduce joint swelling and inhibit the production of TNF- α and IL-1 β prominently. These results indicate that MMP-responsive PEGylated lipid nanoparticles have the latent capacity to treat RA. At present, there are not many researches on nanomedicine in this part, but research in this area should gradually increase in the future.

3.2.3.4 Other sensitive types

Except for what mentioned above, external stimuli such as temperature, light and ultrasound treatment and other stimuli have been used as exogenous triggers to regulate drug release performance. Among them, temperature is one of the stimuli widely explored in the treatment of RA. It is a commonly used method to design nanomedicine through photothermal effect. For example, Costa Lima et al. integrated MTX and gold nanoparticles into PLGA nanospheres by emulsion diffusion evaporation technology. Under near-infrared radiation, the heat generated by gold nanoparticles raises the temperature of the inflamed area and accelerates the release of MTX, thereby improving the therapeutic effect.

In addition to using light and heat effects, light can also control the release of substances. Si et al. pioneered a new way of thinking. By imitating the human breathing process, they prepared a respiratory micelle (BM) capable of inhaling nitric oxide (NO) and exhaling carbon monoxide (CO). Overproduction of NO is a sign of RA. On the other hand, recent studies have shown that CO has essential anti-inflammatory effects. CO can bind to the heme iron center to inactivate inducible nitric oxide synthase (iNOS). At the same time, it increases the production and activity of heme oxygenase-

1 (HO-1). Based on the former theory, they created a "respiratory platform" in which the respiratory micellar nanoparticles have NO-reactive o-phenylenediamine (PDA) and CO-releasing 3-hydroxyflavone (3-HF) derivatives in the core. It consumes NO by forming a benzotriazole (BTA) part, and 3-HF derivatives are CO donors, which can release CO under visible light irradiation, as it shown in the figure below. Through the dual effects of consuming NO and producing CO, the therapeutic effect is greatly improved.

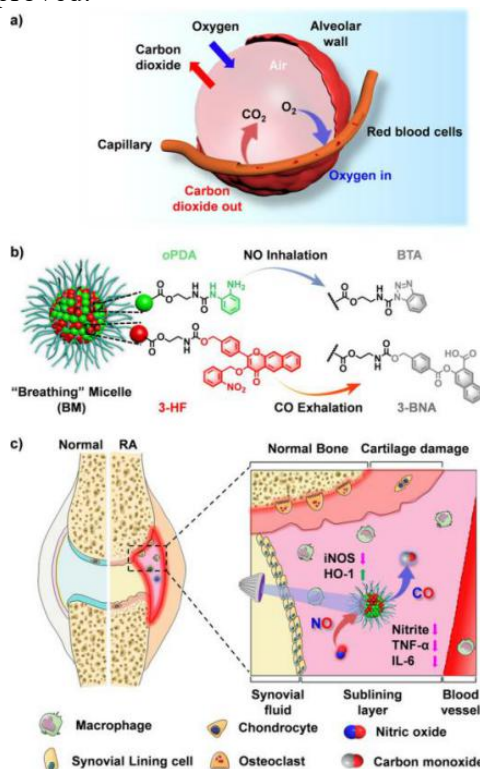


Fig. 5 a) A schematic diagram of inhaling oxygen (O₂) and expelling carbon dioxide (CO₂) in the alveoli. b) The working principle of "breathing" micelles (BM), which inhale NO by converting o-phenylenediamine (oPDA) into benzotriazole (BTA) moieties and pass the light of the 3-HF derivative. Oxygenation exhales CO and forms 3-(benzoyloxy)-2-naphthoic acid (3-BNA). c) BM has a combined anti-inflammatory effect on RA treatment.

Ultrasound technology used to be as diagnose means. However, recent studies have found that ultrasound is emerging as an external trigger in the development of stimulus-responsive nanocarriers. Different ultrasound frequencies are used to control the release of drugs.

Compared with internal stimulation, external ones are more conducive to human manipulation.

4. Conclusion

We have summarized three nano-targeted drugs with targeting strategies. Although passive targeting can improve the solubility and targeting of drugs, these drugs will activate the complement system after entering body, be recognized and eliminated by the monocyte phagocytic system, and weaken the delivery effect of the drug; In the positive targeting strategy, even if the targeting effect is improved, due to the intervention of certain macromolecular components, it may be targeted to normal cells and trigger toxic reactions; in the stimulus response strategy, external stimuli will exhibit insufficient penetration. In order to improve these problems, in the practical cases, various targeting strategies will be used interspersedly: for example, the Jilin University team developed multifunctional nanocarriers that target FA receptors and pH responsiveness, which shows more superiority. Almutairi and others designed a ROS-responsive dextran-drug conjugate (Nap-Dex), equipped with ROS-responsive PBA-modified anti-inflammatory drug naproxen, which interacts with H₂O₂ in an acidic environment. Under the condition of costimulatory nanomedicine, it is more

effective in eliminating ROS, and the levels of pro-inflammatory cytokines IL-6 and TNF α are reduced by 120 times and 6 times separately. In view of extent research, future research will focus more on the design of combination of multiple nanoparticles.

The combination of multiple strategies can improve the targeting and release rate of drugs, but nevertheless other shortcomings of nanomedicine cannot be ignored. The first point is that the production process of most nano-medicine is complicated and the cost is relatively high; the second point is that nano-medicine is difficult to realize the transformation of results, and most drugs are still in the laboratory exploration stage and have not been developed into a medicine; the third point is that it is because the pathogenesis of RA is not yet fully understood, so even if the effect of nano-medicine is perfect, it is only a "treatment of the symptoms but not the root cause". So, we're looking forward to the future, mabe with the development of gene therapy, this situation can be improved.

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