NANOMEDICINES FOR INCREASED SPECIFICITY AND THERAPEUTIC EFFICACY OF RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA), the most frequent chronic inflammatory autoimmune disease, can lead to pain, bone and articular destruction, and limb deformity and impairment, with great impact on the activities of daily life. Several drug modifiers of the inflammatory process have been used in the treatment of the disease, all with specific patient targets and indications. However, the side effects are a frequent cause of undertreatment and non-adherence. To promote better compliance with the therapy, drug researchers have been trying to develop a new carrier of the immunomodulated molecules to increase their concentration in the target cell (mostly synovial), avoiding side effects for organs that are not targeted, as well as providing an easier manner of administration. The research results from animal models are promising and the clinical applications will show if these results are similarly impressive. This paper aims to explain the major applications of nanomedicine in RA treatment and diagnosis. The use of nanocarriers able to act as a diagnostic imaging agent and targeted drug delivery system, simultaneously, also known as nanotheranostics, can allow an improved efficacy and safety pharmacological profile, earlier detection, and thither monitoring of the disease.[1] Commercial successes of RA active targeting of nanomedicine and products under development will be revised.

Keywords: Intracellular delivery, nanomedicine, rheumatoid arthritis (RA), safety, surface modification, targeted delivery, theranostics.

Rheumatoid arthritis (RA) is one of the most common and severe autoimmune diseases related to the joints.[2,3] Regrettably, the RA inflammatory process remains puzzling, and finding effective therapies for the disease, as well as a new means for its early diagnosis, has been a daunting task. It is characterised by chronic inflammation of the synovial membrane, which often leads to destruction of articular cartilage, periarticular bone erosion, and permanent deformities. Consequently, more macrophages, lymphocytes, and fibroblasts are activated and the RA inflammatory process remains.[3-5] Thus, macrophages play a pivotal role in the features and progress of RA, and effective diagnosis and therapy may include the ability to target these cells.

Currently, the main target of RA therapy is to control the inherent inflammatory response, alleviate pain, and avoid structural bone damage and deformities. Several therapeutic options have been used to manage and slow the progression of the disease, which include the use of sulfasalazine,

hydroxychloroquine, corticosteroid-related drugs, or methotrexate (MTX), a first-line, disease-modifying anti-rheumatic drug.[6,7] MTX is widely used due to its satisfactory safety profile, efficacy, and low cost. It is an analogue of folic acid that disrupts cellular folate metabolism by inhibiting its target enzyme, dihydrofolate reductase.[7-9] Still, there is a lack of specificity for MTX and/or other similar drugs.

More recently, biologic agents were developed to target components of the immune response involved in RA, including proinflammatory cytokines and immune cells. Most of these biologicals include cytokine antagonists (tumour necrosis factor [TNF] blockade, interleukin-1 receptor blockade, and interleukin-6 receptor inhibitors), B-cell depleting agents, T cell co-stimulation modulators, and kinase inhibitors.[10] Despite their efficacy, biologicals are also associated with invasive routes of administration, stability issues, and significant adverse effects, which may limit their use. Recent developments in the understanding of inflammation have led to an increased interest in the use of nanomedicine in the treatment of RA, overcoming pharmacokinetic and pharmacodynamic issues related to the classical intravenous formulation, such as poor solubility of active molecules in physiological fluid or premature degradation of drugs.

Nanotechnology is a multidisciplinary research field, concerning the study of devices usually ranging from 1–100 nm, though in larger systems this is ≤1,000 nm. As a consequence of its vast success on the development of biocompatible colloidal systems, such as nanoparticles, nanocapsules, micellar systems, and conjugates, nanomedicine has thrived and is now providing new possibilities for the use of nanomaterials in medical applications for drug delivery and tissue regeneration.[11-13] In this technology, unique phenomena enable novel applications as nanosystems have new properties, such as large surface-volume ratio, surface charge, and small and controlled size. Nanoparticles built with biodegradable, biocompatible biomaterials have gained attention from the scientific community, which is a result of their controlled and sustained release properties, subcellular size, and feasibility of production.[14] The most commonly used polymeric nanoparticles include poly(lactic acid), poly(lactic-co-glycolic acid) (PLGA), poly-ε-caprolactone, poly(alkylcyanoacrylates), and chitosan, while proteins such as albumin and gelatin have been widely explored. Lipid-based nanocarriers have also been proposed. Colloidal drug delivery systems have been described as reducing systemic side effects and maintaining of appropriate drug concentration in the required place.

Nanomedicine may also offer new opportunities to combine diagnosis and therapy in a single approach. Improved theranostics processes (combining diagnostics and therapy) are being studied to develop new means to diagnose, fight, and follow disease. The release and action of anti-rheumatic drugs may be enhanced and controlled, potentially without injuring healthy tissues and organs, while simultaneously providing a non-invasive and specific imaging tool for RA. A theranostic nanosystem combines non-invasive diagnosis and treatment, with the possibility of monitoring real-time drug release and distribution, thus predicting and validating the effectiveness of the therapy. This optimised algorithm treatment to each patient can be specifically performed, achieving the call for personalised nanomedicine.[15]

Conventional radiographs, magnetic resonance imaging (MRI), and ultrasonography are used to measure joint damage in patients with RA. MRI is especially sensitive in detecting early pathology due to its duality in assessing both inflammatory and structural lesions.[16] In the context of MRI-based diagnosis, superparamagnetic iron oxide nanoparticles (SPION) are most frequently used.[17] Despite their intrinsic diagnostic capabilities, these magnetic nanoparticles can be co-encapsulated with a therapeutic agent into other nanoparticles, such as US Food and Drug Administration (FDA)-approved polymer PLGA.[18] SPION can also be associated with other imaging tools, such as optical/fluorescence or offer additional targeting approaches. For example, they can be guided to target sites using external magnetic fields or heated by an external source to provide hyperthermia.[19]

At present, marketed products based on nanomedicines are useful tools for the diagnosis and treatment of prominent diseases. There are different examples of nanomedicines with potential application in RA therapy based on different kinds of nanoparticulate systems, as recently revised.[10] However, the clinical translation of such nanomedicines into clinical trials or products is still in its infancy. There are no clinical trials enrolling RA and nanoparticles considering the new biologics (e.g. adalimumab) as matrix nanostructures. A significant number of the studies have their proof of concept in disease animal models

resembling the RA condition, expecting a future translation into humans.

Additionally, further surface functionalisation of nanoparticles to impart precise biological functions is considered a promising approach in drug delivery. It holds the potential to play an important role in personalised medicine, namely in addressing clinically unmet needs that are highly variable among patients.[20] The modification of the surface of particles provides physicochemical and biological characteristics that can radically change the properties of a material. With RA, nanomedicines can be designed to remain in blood circulation for a longer time, may be surface-conjugated with ligands to facilitate active targeting, can be tailored to macrophage uptake or targeted to certain receptors, and pass through interendothelial cell gaps in diseased tissue. Several active targeting strategies for nanoparticles in RA have been proposed in recent years. In an active targeting approach, ligand molecules, such as antibodies and specific adhesion molecules, attach to the surface of nanoparticles, which can increase the probability of binding to membrane receptors upregulated in RA key local effector cells. These are mainly macrophages or monocytes and fibroblasts.[21] They are local and systemic amplifiers of RA severity and perpetuation is maintained by means of cytokine and cell contact-mediated activation of neighbouring inflammatory cells.[22] Cellular surface proteins, such as CD11b, CD90, HLA-DR, and CD64 receptor of inflammatory macrophages,[23] have been recognised as highly overexpressed antigens in RA-affected synovial tissue, which may be promising biomarkers for novel targeted drug delivery and diagnosic approaches in RA.[23-26] Other agents involving the folate receptor-β of rheumatoid arthritis synovial macrophages,[27,28] or intercellular cell-adhesion molecule-1[29] have also been described. Functionalised nanomedicines may suffer from intrinsic biological variability due to pathology and physiological variations, and possess a limited landscape as far as scale-up production is concerned. Being more elaborate in terms of production steps, due to the surface conjugation of ligand moieties, means that additional steps in the production chain may be required, which makes the manufacturing process more complex.

In a recent study for the treatment of inflammatory arthritis, using a well-established and clinically relevant rat model of adjuvant-induced arthritis, different nanomedicine formulations of dexamethasone, including liposomes, micelles, slow-releasing, and fast-releasing polymeric prodrugs were evaluated.[30] It was found that after a single intravenous injection, formulations with the slower drug release kinetics maintained longer duration of therapeutic activity than those with relatively faster drug release kinetics, resulting in improved joint protection. This finding will be instructional in the future development and optimisation of nanomedicines for the clinical management of RA.

In order to target nanoparticles in RA therapy, hyaluronic acid-MTX conjugates that can specifically bind to CD44 on the inflammatory cells and release MTX in an inflamed tissue of RA have been developed.[31] Those nanoconjugates were taken up by activated macrophages more efficiently than free MTX through binding CD44 and hyaluronic acid. *In vivo* biodistribution tests confirmed that hyaluronic acid–MTX conjugates were selectively accumulated into the inflammatory joint site of the collagen-induced arthritic mouse, improving clinical arthritis indices and reducing proinflammatory cytokines and pathogenic immunoglobulin G.[31] It was also confirmed that the use of hyaluronic acid as a nanocarrier increases the residence time of MTX in affected joints.[32] Hyaluronic acid-MTX conjugates exerted anti-arthritic effects in two different models of arthritis, with a wider therapeutic window than oral MTX.

The use of gold compounds for the treatment of RA is well-established, although the mechanism of action of chrysotherapeutic agents is not very clearly understood. Clinical protocols have been proposed by injecting patients with regular-sized gold compounds to reduce inflammation. Attempts have been made to expose macrophages to gold nanoparticles and compare them with gold complexes, which have resulted in a significantly greater uptake of gold without significant cytotoxicity towards macrophages.[33] These results support the potential of these colloidal systems as anti-RA agents. When combining the delivery of gold nanoparticles with MTX to the region of inflammation in collagen-induced arthritic mice, the retention of nanoparticles was enhanced under the external magnetic field,[34] resulting in enhanced therapeutic effects with an MTX dosage of just 0.05% compared to free MTX therapy for the treatment of RA.[34]

The use of small interfering RNA (siRNA) has shown therapeutic effects in diverse disease models by silencing the gene responsible for the

defects, including RA.[35] In order to enhance the therapeutic efficacy of siRNA, incorporation of siRNA into nanoparticles has shown improved target ability.[36] Despite the lack of research in this area, the delivery of siRNA entrapped into nanoparticles has been able to downregulate fundamental protein molecules involved in the RA physiologic process, such as Notch1[35] or TNF-α.[37] In the future, particular attention may be given to Notch1, a signalling receptor, overexpressed in synoviocytes that has been shown to contribute to TNF-α-induced proliferation. Such targeting of siRNA delivery systems in siRNA-nanoparticles has been shown to be a superior effective RA treatment compared to free siRNA by suppressing protein signalling pathways without any undesirable severe toxicity.

Insights on multifunctional anti-CD64 mAb-modified nanoparticles for the combined delivery of MTX and iron oxide nanoparticles (SPION) has also been proposed.[38,39] Polymeric nanoparticles have the potential to provide a new theranostic approach for RA management. When considering the potential delivery methods for nanomedicines to target RA, intravenous administration is gaining the most attention, both preclinically and clinically. The increased interest in intravenous delivery is not surprising given that nanoparticles delivered systemically have direct access to nearly all parts of the body, including joints, and thus have the most potential to influence clinical care.[40] However, systemically delivered nanoparticles still face exceptional challenges with regard to delivery, clinical translation and regulation. If delivery aspects are easy to overcome, clinical translation and integration relies on a consistent and reproducible product. On the other hand, the ability of nanomedicines to permeate into and/or be retained in the inflamed joint after intra-articular administration has proven to be beneficial in improving RA therapy while reducing systemic exposure of patients to potentially toxic anti-arthritic drugs.[41] This is an alternative worth exploring, considering the controlled and sustained drug delivery properties that nanoparticles may provide in the synovial fluid.

Nanoparticles used in preclinical studies are almost entirely synthesised in small batches and scaling up production for the synthesis of large quantities is not always possible. There is a constant and increasing need to produce consistent and highly reproducible formulations prior to the clinical trial stage.[40] Depending on the methodology of production, scale-up may require additional modifications that most pharmaceutical companies are not prepared to implement. When compared to free drugs, it should also be recognised that nanoformulations will raise the overall cost of production, due to the above-mentioned additional steps. Still, the cost-benefit ratio is clearly well-balanced.

Lastly, from the regulatory perspective, nanomedicine products are mostly administered within a conventional regulatory framework by regulatory agencies. However, additional expert evaluations are necessary to confirm the quality, safety, and efficacy of nanotherapeutics because of their complexity, which may be established in specific guidelines and methodologies, on a case-by-case basis. For regulatory decision-making, it will be imperative to define critical product attributes predictive of product performance *in vivo*.

In summary, there is increasing interest in nanomedicines for the management of RA, with researchers looking for safer and more efficient treatments compared to current medicines. The implementation of nanomedicines, with the potential to control the release kinetics of drugs due to the tailored degradation properties of nanoparticle matrix, can be explored to adjust the therapeutic concentration of anti-RA drugs. Biocompatible and biodegradable materials can be used for the formulation of active payloads, also incorporating scientific knowledge from other drug development processes. In contrast, the optimisation of targeted systems is less straightforward, as mentioned before, regarding the additional steps in the manufacturing process. In the future, it is foreseen that the development of new ligand moieties attached to the surface of nanoparticles may drive those systems to the active sites. Such innovative ligands may enhance the current knowledge of cells involved in the RA process, and increase understanding of the pathophysiology of RA. Additional advantages may be expected by co-administration of different drugs in synergic treatment protocols, with formulation incompatibilities, as nanoparticles offer physical stability during storage. Also, the possibility of formulating more than one drug, with different physical-chemical properties in the same nanoparticulate system, may offer patients less painful treatment regimens and, in the long term, help to overcome the high cost of biologics.

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