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# Biomaterial-based immunotherapeutic strategies for rheumatoid arthritis

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## Abstract

Rheumatoid arthritis (RA) is an extremely painful autoimmune disease characterized by chronic joint inflammation leading to the erosion of adjacent cartilage and bone. Rheumatoid arthritis pathology is primarily driven by inappropriate infiltration and activation of immune cells within the synovium of the joint. There is no cure for RA. As such, manifestation of symptoms entails lifelong management via various therapies that aim to generally dampen the immune system or impede the function of immune mediators. However, these treatment strategies lead to adverse effects such as toxicity, general immunosuppression, and increased risk of infection. In pursuit of safer and more efficacious therapies, many emerging biomaterial-based strategies are being developed to improve payload delivery, specific targeting, and dose efficacy, and to mitigate adverse reactions and toxicity. In this review, we highlight biomaterial-based approaches that are currently under investigation to circumvent the limitations of conventional RA treatments.

**Keywords** Rheumatoid arthritis · Autoimmunity · Biomaterials · Nanomedicine · Drug delivery

## Introduction: a brief background, etiology, and epidemiology of rheumatoid arthritis

Rheumatoid arthritis is a debilitating autoimmune disease characterized by chronic inflammation and immune cell infiltration of the joint synovium leading to articular bone and cartilage destruction [1]. Development of rheumatoid arthritis (RA) is complex and driven by various layers of immunity working in concert to promote joint pathology [2]. The pathogenesis of RA is poorly understood, though some clues help demystify the process. Several risk factors for RA have been identified. Sex seems to play a determinative role, as women are 3 times more likely to develop RA than men. Additionally, familial penetrance of RA is up to 50% [3]. Several studies have also linked poor quality of diets to RA. Patients with RA tend to eat more saturated fats and sugars and fewer fruits, vegetables, and whole grains [4]. The most implicated genes conferring risk of RA are (1) the shared epitope (SE), which is encoded by a set of alleles located within the major histocompatibility (MHC) locus, and (2) the

PTPN22 polymorphism [3, 5], which encodes an abnormal form of lymphoid protein tyrosine phosphatase, an enzyme that inhibits T cell activation [6]. Antigen-presenting cells (APCs) harboring the SE mutant possess enhanced efficiency for presenting citrullinated peptides in their MHC class II molecules [7], whereas T cells harboring the PTPN22 mutant have impaired self-regulation and are prone to remaining in an active state [8]. Additionally, the wild-type form of PTPN22 normally inhibits intracellular citrullination. However, the PTPN22 polymorphism is unable to do so, resulting in hypercitrullination within immune cells [9]. For the most part, environmental risk factors largely introduce states of inflammation at the mucosal level [10]. For example, smoking, obesity, air pollution, and periodontitis have all been implicated in the development of RA [3, 10], and the presence of both genetic and environmental risk factors significantly increases the likelihood of RA [3]. There is currently no cure for RA, and it continues to be a major health burden affecting ~1% of the population [11]. If left unchecked, the chronic systemic inflammation may cause extra-articular complications in the brain, liver, lungs, and heart [2]. Mortality rates in patients with RA is increased by 1.5 times (compared to those without RA), largely due to cardiovascular and lung disease likely arising from or complicated by systemic inflammation [12]. Moreover, those afflicted with RA suffer

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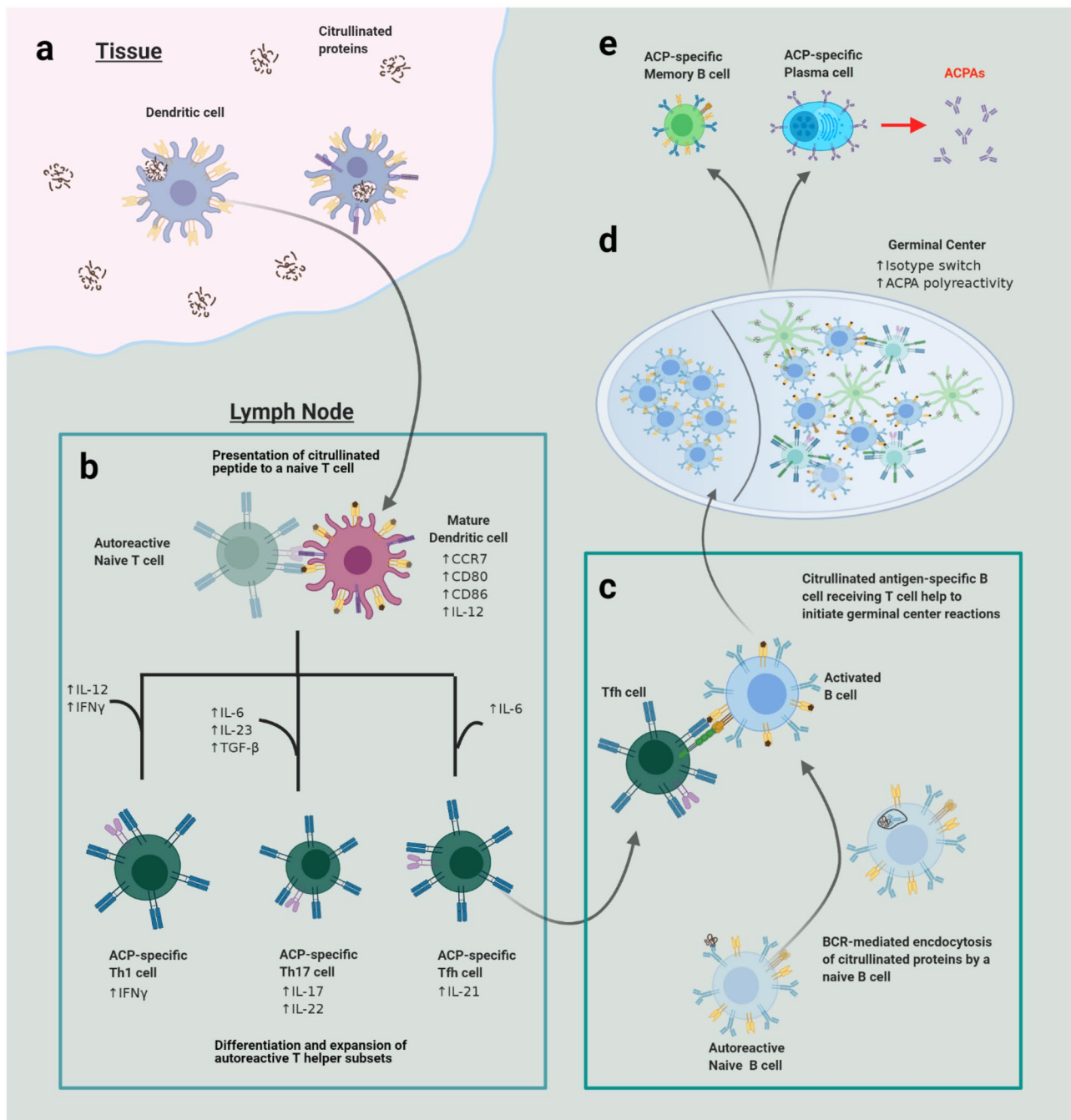
tremendous economic costs due to expensive treatments, impaired work performance, disability, and early retirement [13]. Further exacerbating their situation are enormous indirect costs through psychological stress, deformity, and progressive decline of self-worth [14]. Current treatment options are suboptimal as they vary in efficacy [15]. Long-term methotrexate is the prescribed anchor drug for RA. However, the PREMIER study, a multicenter, randomized double-blind clinical study, revealed that only 46% of RA patients taking methotrexate experienced a 50% improvement in RA symptoms [16]. Therefore, methotrexate is often combined with other biologics in pursuit of symptom alleviation and remission [15]. Unfortunately, these treatments are accompanied with a multitude of side effects, making adherence to a dosing schedule challenging [17]. An overarching strategy used to treat RA is disruption of inflammatory mediators using targeted drugs [15]. However, these drugs render the patient vulnerable to infection [17], and also are associated with increased risk of cancer [18, 19], which may be through impairing immunosurveillance of oncogenic precursors [20]. Additionally, major concerns have recently arisen regarding the use of immunosuppressants amid the SARS-CoV-2 pandemic [21–23]. As such, there is a critical need for uncovering new strategies for the treatment of RA.

## RA immunopathogenesis

The quintessential biomarkers for RA are the presence of two autoantibody types: (1) rheumatoid factors (RFs), which are IgM, IgA, or IgG antibodies against the fragment crystallizable (Fc) region of IgG antibodies, and (2) anti-citrullinated protein antibodies (ACPAs), which consist of IgM, IgA, or IgG antibodies that bind peptides which have undergone citrullination, a post-translational modification mediated by the enzyme peptidylarginine deiminase (PAD) [24, 25], though the absence of these autoantibodies does not exclude you from developing RA (seronegative RA), which accounts for about one-third of RA cases [26]. Intriguingly, naïve B cells specific for citrullinated peptides have been detected in the periphery prior to RA manifestation [27], which suggests that autoreactive B and T cells are able to evade central and peripheral tolerance [28]. In support of this, longitudinal studies have revealed that RF and ACPAs can be present in the circulation years presaging the manifestation of RA [29, 30], and the presence of both autoantibody types are associated with elevated levels of inflammatory cytokines and accelerated onset of RA [31], suggesting induction of autoantibodies is an early step in the gradual progression towards development of RA and may require additional factors to trigger disease. This window characterized by presence of autoantibodies and absence or minimal joint inflammation is described as the pre-RA stage

[32]. This lag between the detection of RFs and ACPAs and onset of RA may be explained by the evolution and accumulation of multiple ACPA reactivities, which requires time for endogenous antibody maturation and epitope-spreading mechanisms to occur [27, 33, 34]. Additionally, ACPAs develop against various peptide targets including  $\alpha$ -enolase, fibronectin, type II collagen, fibrinogen, vimentin, and histones [35], and also exhibit cross-reactivity between citrullinated peptides [36]. Taken altogether, immune cells working cooperatively to accumulate antibodies against various citrullinated epitopes may be the factor that tips the scale in favor of RA onset.

In the periphery, dendritic cells (DCs) harboring the SE variant possess enhanced ability to present citrullinated peptides within their MHC class II molecules [37, 38], and upon activation, the chemokine receptor CCR7 is upregulated to facilitate DC migration towards lymphoid organs rich in CCL19 and CCL21 to initiate an adaptive immune response [39] (Fig. 1(a)). Within the synovium, DCs exposed to inflammatory cytokines such as interferon gamma (IFN- $\gamma$ ) respond by making high levels of interleukin (IL)-12, which, in turn, promotes a Th1 response [40]. Critical to RA pathology, DCs drive autoreactive T cell expansion through citrullinated peptide presentation, co-stimulation, and cytokine production (Fig. 1(b)) [35]. Following this, autoreactive B cell activation is able to receive T cell help to initiate germinal center reactions to produce ACPA-secreting plasma cells (Fig. 1(c)–(e)). The joints possess an abundance of citrullinated epitopes, which provide a natural propensity for ACPAs to accumulate in articular tissue and induce pathology [24]. This stage of established disease, denoted as “early RA”, is characterized by immune dysregulation and inflammation within the joints [32]. Within the joints, following ACPA ligation and RF deposition, immune complex (IC) formation activates macrophages, osteoclasts, neutrophils, and DCs through FC $\gamma$ RIII and complement receptors to initiate an inflammatory response [24]. In comparison to a healthy joint normally devoid of immune cells (Fig. 2a), synovitis in a rheumatic joint is perpetuated by infiltration and coordination of immune cells [40]; synovial infiltration of T helper (Th) 17 cells, Th1 cells, B cells, macrophages, neutrophils, and DCs perpetuates an intensely inflamed environment as long as the antigen is present, eventually leading to adjacent bone and cartilage erosion [2, 35, 39, 41]. Upon cytokine stimulation, activated synoviocytes release the chemokines CCL2 and CX3CL1 to recruit monocytes from circulation and polarize them towards an M1 phenotype or differentiation into osteoclasts [42]. M1 macrophages further promote inflammation through production of the pro-inflammatory cytokines, including tumor necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and IL-12, as well as [43, 44] granulocyte–macrophage colony stimulating factor (GM-CSF) and monocyte chemoattractant protein-1 (MCP-1)

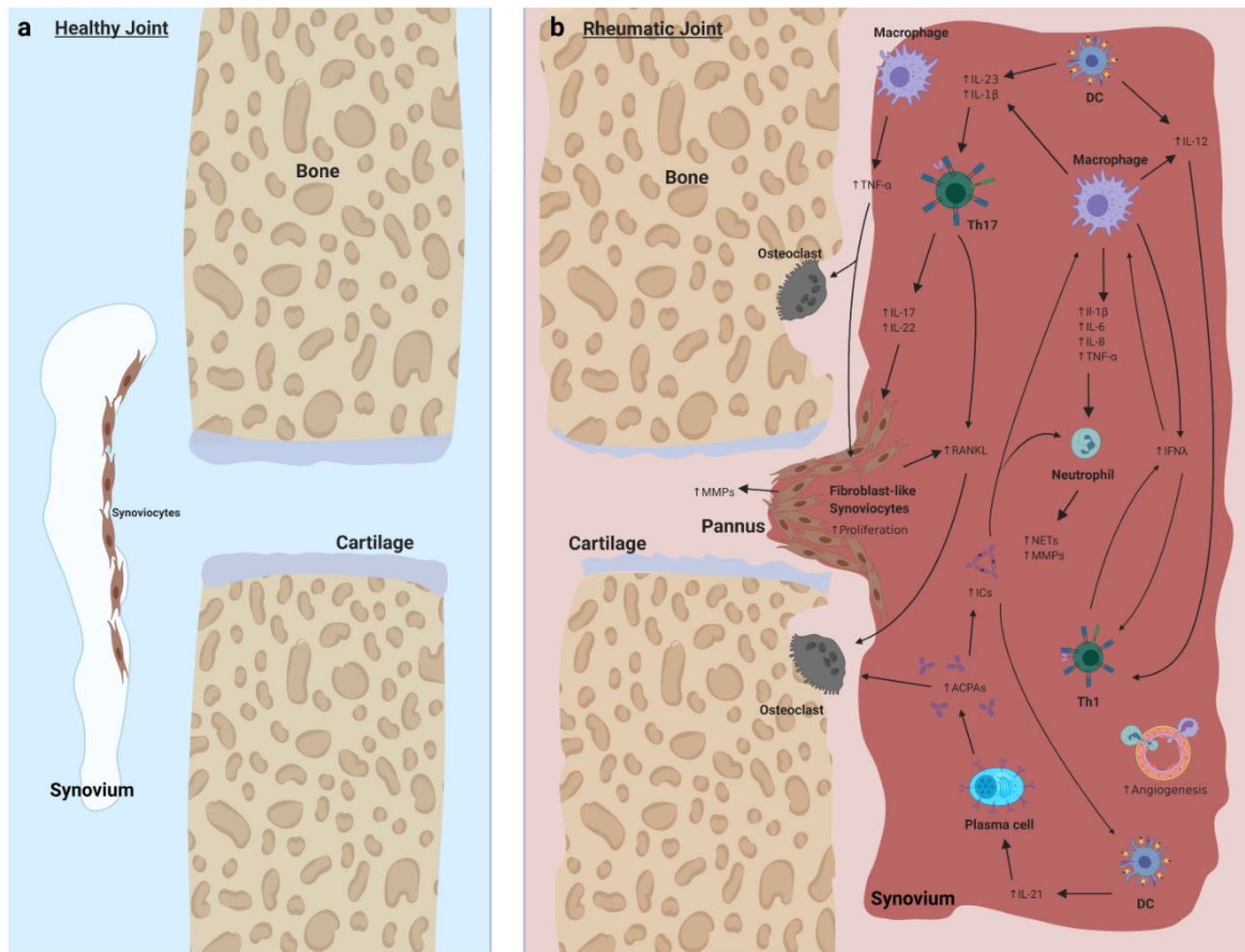


**Fig. 1** Immunopathological induction of anti-citrullinated protein antibodies. **a** Dendritic cells encounter and phagocytose citrullinated proteins in the tissue, and process them into citrullinated peptides presented within MHC II molecules. **b** The mature dendritic cell migrates to the lymph nodes to activate the citrullinated peptide-specific naive T cell, inducing differentiation and expansion of citrullinated peptide-specific Th1, Th17, and Tfh subsets. **c** A naive B cell undergoes B cell receptor-mediated uptake of citrullinated

protein, and processes them into citrullinated peptides presented within MHC II molecules. The newly activated B cell then receives co-stimulatory help from a citrullinated peptide-specific CD4 T cell to initiate germinal center reactions. **d** Directed evolution of the B cell receptor within the germinal center increases its affinity to citrullinated proteins. **e** Differentiation of citrullinated protein-specific memory B cells and ACPA-secreting plasma cells

[43, 44], while upon IFN- $\gamma$  stimulation, Th1 effector cells produce IFN- $\gamma$  and TNF- $\alpha$  [40]. TNF- $\alpha$  helps activate T cells, synoviocytes, and stromal cells, which in turn release

receptor activator of nuclear factor-kB ligand (RANKL), the primary growth factor that drives osteoclastogenesis [45] and osteoclast-mediated bone resorption [42]. Additionally,



**Fig. 2** Immunopathogenic mechanisms within a rheumatic joint. **a** A healthy joint is devoid of immune cell infiltrates. **b** An inflamed rheumatic joint. ACPA ligation and ensuing immune complex formation activate local innate cells to produce cytokines and chemokines, resulting in migration and activation of additional immune cells from

the circulation. TNF- $\alpha$  and RANKL induce osteoclast-mediated resorption of bone. IL-17, IL-22, and TNF- $\alpha$  induce proliferation of fibroblast-like synoviocytes and pannus formation. MMP secretion from neutrophils and fibroblast-like synoviocytes degrades cartilage

activated Th17 cells secrete IL-17 and IL-22, which upregulate RANKL expression in synoviocytes [46, 47]. Citrullinated actin and vimentin are expressed on the cellular surface of DCs, and ACPA ligation promotes transdifferentiation into osteoclasts [48]. Osteoclasts express vimentin, and ACPA ligation upregulates TNF- $\alpha$  production [49]. Furthermore, the inflamed joint is abundant in ACPA-secreting plasma cells, B cell aggregates [50], and activated T cells, which secrete CXCL13 and CCL20 to recruit B cells to the synovium [50, 51]. Moreover, functional germinal centers have been detected within the synovium itself [50], which provides a local source of newly minted autoreactive plasma cells. Neutrophils in the circulation become primed and infiltrate the synovium by sensing high levels of IL-8, complement component 5a (C5a), and leukotriene B4 (LTB4) [52]. Upon exposure to IL-17a, TNF- $\alpha$ , and ACPAs within the

joint space, neutrophil extracellular trap (NET) extrusion is strongly induced, releasing citrullinated antigens from within the neutrophil into the synovium [53]. This NET formation in turn activates local synoviocytes to produce IL-6 and IL-8, further activating neutrophils [53]. Activated synoviocytes also secrete matrix metalloproteinases (MMPs), which contribute to the remodeling of adjacent cartilage [54]. Neutrophils also participate in cartilage breakdown through secretion of MMP-8, MMP-9, elastase, cathepsin G, and proteinase 3 [54]. There is emerging evidence that distinguishes late RA from early RA by the enhanced contribution of synoviocytes to disease, which may explain why immunotherapy is less successful at this stage [32]. In late stage RA, these synoviocytes display a bona fide transformed phenotype towards fibroblast-like synoviocytes and are highly proliferative and also resistant to apoptosis [32].

Within the joint, these pathological immune cell and stromal cell interactions are descriptive of established RA and are depicted in Fig. 2b.

### Standard clinical practice for RA therapy and associated adverse effects

Upon clinical diagnosis of RA, the standard guideline calls for treatment with a disease-modifying anti-rheumatic drug (DMARD) which include hydroxychloroquine, sulfasalazine, leflunomide, and methotrexate [55]. The first drug regimen typically defaults to methotrexate in combination with glucocorticoids (GCs) such as prednisone or prednisolone [56]. The mechanism of methotrexate is, putatively, through the elevation of anti-inflammatory adenosine to paracrine levels [57, 58], which inhibits neutrophil activation, promotes macrophage polarization towards an M2 phenotype, dampens T cell activation, promotes regulatory T (Treg) cell induction, and promotes endothelial cell tight junction integrity [58]. Additionally, methotrexate is a folate inhibitor, which results in purine depletion, cell cycle arrest, and eventually apoptosis in rapidly proliferating cells [59]. Glucocorticoids are robustly anti-inflammatory through dampening of innate immune cell signaling and promoting of the apoptosis of lymphocytes [60]. While effective, great caution must be considered when prescribing GCs due to side effects such as loss of bone density [61], insomnia, depression [62], and also long-term toxicities [63]. If the prescribed therapy is successful in reducing RA symptoms, then treatment is tapered until remission is sustained at the smallest dose possible [64]. However, if the therapy is ineffective, then the treatment strategy is continuously modified by adding or substituting available biological or targeted synthetic DMARDs until remission is achieved, which is more likely with combinatorial therapies [56]. The biological DMARDs consist of biologically manufactured molecules that have been harnessed for their effect in treating RA; these include TNF inhibitors (such as adalimumab, infliximab, and etanercept), the immune checkpoint protein cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mimic (abatacept), anti-CD20 antibody (rituximab), anti-IL-6 antibodies (tocilizumab) [35], and histone deacetylase (HDAC) inhibitors (trichostatin A, givinostat, or valproic acid) [65]. Inhibition of TNF- $\alpha$  removes a pan-activating cytokine that is highly elevated in the inflamed synovium [66]. While the toxicity of TNF inhibitors is largely absent, common side effects include injection site reaction as well as increased risk of infection [67]. The CTLA-4 mimic, abatacept, works by competitively binding to co-stimulatory molecules, CD80 and CD86, on the surface of APCs, which prevents the T cell CD28 co-receptor from docking and providing necessary T cell activation signals [68]. Patients receiving abatacept

commonly experience headaches and nausea [68], and more severe adverse effects such as serious infection, cardiovascular events, cancer, or death occur at 6.4/100 patient-years [69]. The anti-CD20 antibody, rituximab, works by depleting B cells and, thus, the source of ACPAs [70]. With rituximab, severe adverse effects of infection, cardiovascular events, cancer, or death also occur at a rate of 7.3/100 patient-years [69]. Targeted, synthetic DMARDs are molecules that have been fabricated to target specific pathways in RA pathology, for instance, the Janus kinase (JAK) inhibitors — tofacitinib and baricitinib [71]. Many inflammatory cytokines that contribute to RA pathology such as IL-6, interferons (IFNs), granulocyte colony-stimulating factor (G-CSF), and GM-CSF rely on the mediator proteins, JAK, to transmit intracellular cascades required for their production; thus, JAK inhibition serves to suppress inflammatory cytokine levels [72]. Long-term administration of tofacitinib revealed a rate of serious adverse effects (infection, cardiovascular events, cancer, or death) at 6/100 patient-years [69]. Depending on the disease activity measure used to determine remission, a multinational cross-sectional meta-analysis revealed that remission was achieved at rates between 8.6 and 19.6% in RA patients [73]. The rate of relapse (1) for ongoing treatment was 15.8%; (2) for remission and 50% dose reduction, 38.9%; and (3) for stopping treatment, 51.9%, as determined in a multicenter, randomized controlled, parallel-group phase 3 trial [74].

### Biomaterial-based immunotherapies for RA

Existing drugs inherently possess limitations in terms of half-life, dosage, efficacy, and toxicity. For instance, the half-life of methotrexate is approximately 6 h [75]. The incorporation of biomaterials into drug design, or rather drug delivery technology, blunts conventional drug limitations, permitting augmentations in a myriad of ways. Biomaterials are any materials not considered food or drugs that come into contact with tissue [76] and are being routinely investigated in many therapeutic applications including cardiovascular disease [77], neurodegenerative disease [78], cancer [79], vaccines [80], and autoimmunity [81–83]. Using biomaterials to engineer drug delivery systems across different scales offers numerous advantages. For example, encapsulation of the drug protects it from environments that may cause degradation [76] such as changes in pH or exposure to enzymes. This also extends the drug half-life, which allows for less frequent and smaller doses, thereby lowering the risk of toxic side effects [76]. Manipulation of physicochemical properties of the biomaterials also allows extensive versatility in engineering particulate systems. For instance, passive targeting describes a circumstance where nano- or microparticles can accumulate in target tissues through their innate characteristics [84].

Nanocarriers smaller than 5  $\mu\text{m}$  are readily engulfed and thus passively target and accumulate within phagocytes [84]. Moreover, nanocarrier uptake by DCs also tend to accumulate within lymph nodes (LNs) due to DC ability for intranodal trafficking [84]. Another example of passive targeting is through exploitation of the enhanced permeability retention (EPR) effect [85]. The EPR effect describes a phenomenon where nanoparticles tend to accumulate within tissues with leaky vasculature, such as sites of inflammation, or within tumors [85]. Membrane surfaces of the nanocarriers can also be customized through ligation of antibodies, peptides, and receptors, or incorporation of environmentally sensitive bonds [86] to facilitate active targeting of specific tissue compartments [84]. Nanocarriers can also function as drug depots with programmable release kinetics [84]. In this review, we will discuss the employment of liposomes, nanoparticles (NPs), microparticles (MPs), scaffolds, and hydrogels as versatile drug carriers as well as systems to directly modulate immune responses for treating RA.

Current therapeutics for RA fall prey to acute and chronic toxicities and many other adverse effects [17]. Utilizing liposomes as a drug carrier helps to mitigate these unfavorable outcomes [87]. Liposomes as drug carriers boast many advantages with their poor immunogenicity, versatility, and lack of toxicity [88]. In addition, by nature of hydrophobic interactions, the liposome constituents self-assemble into particles with the lipid bilayer segregating the internal enclosures into a lipophilic region encircling an internal lipophobic core, allowing incorporation of both lipophilic and lipophobic drugs and ease of combinatorial delivery [89, 90]. Discovery of polyethylene glycol (PEG) has also served to increase circulatory retention of liposomes by mitigating off-target engulfment [91]. Additionally, the liposome surface is modifiable, as charge alterations and functionalization with antibodies, peptides, and ligands have all been incorporated to improve specific targeting [90]. It follows that liposomes have garnered considerable interest in improving RA therapies. Ren et al. [92] have comprehensively characterized optimal liposome parameters that favor accumulation within the inflamed synovium. The group fabricated different liposomes with altered surface charges, diameters, PEG chain length, and lipid composition, and tested the biodistribution of different formulations via intravenous (i.v.) injection in the collagen-induced arthritis (CIA) mouse model [92]. They determined that a 100-nm, slightly negative liposome composed of 10% 5-kDa PEG chains yielded the highest accumulation within the diseased joint [92]. The data obtained in this study serve as a valuable reference in fabrication of liposomes for articular targeting. Largely, liposomes have been investigated as vehicles for delivery of anti-rheumatic drugs to sites of inflammation. To this end, several groups have demonstrated encapsulation of bioactive molecules within liposomes is an effective

strategy for enhancing articular targeting and potency. For example, dexamethasone [93, 94], glutathione [95], and tofacitinib [96] have been encapsulated within liposomes and shown to be more efficacious in comparison to the unencapsulated drugs in various models of inflammatory arthritis [93, 95–97].

Harnessing endogenous immunologic pathways to treat rheumatic disease is one strategy that has gathered significant interest of late. Nano- and microparticles are well suited for various immunomodulatory applications by design and offer a myriad of benefits. The size of the particles, which ranges from nanometers to microns, allows for both active and passive targeting to sites of interest [84], and also alters the rate of particle degradation to modify release kinetics to be either slower or more rapid [98]. They are uniquely equipped to modulate immune cell function due to their aptitude for direct cytosolic or surface delivery [84, 99]. Notably, cytosolic delivery of a peptide within an APC allows for endogenous biomachinery to cross-present the peptide within MHC class I molecules [100, 101]. This allows for encapsulation of a target peptide within a particle that will promote presentation within both MHC class I and class II molecules of an APC, allowing for modulation of both CD8 and CD4 T cells, respectively [100]. Moreover, the potential for customizability is vast. Altering the material used for fabrication of NPs and MPs can bestow dynamic characteristics to the drug carrier system that allows for targeting of different environmental cues or specific disease states [86], or even potentially conferring intrinsic characteristics into the particle itself [102]. For example, many gut-derived molecules exert immunosuppressive effects and are garnering interest for their potential to be fabricated into particles that possess tolerance-inducing effects [102], and poly-conjugated aspirin particles have been used to confer innate anti-thrombus activity into the particle itself [103]. Additionally, particles that incorporate pH-sensitive bonds such as aliphatic monomers incorporating cleavable acetal links can facilitate preferential cargo delivery into specific environments where the pH is acidic [86]. Other environmental attributes can be targeted for precision-based delivery of the payload through incorporation of bonds that are sensitive to various environmental cues, such as temperature, enzymes, light, and hypoxia [86]. A comprehensive review encompassing many different stimulus-responsive biomaterials can be found here [86]. Additionally, the membrane surface of NPs and MPs can be functionalized with a variety of ligands, such as peptides, receptors, or antibodies, further allowing precise delivery to specific immune cell subsets or tissues [83, 84, 104]. Microparticles can also act to provide safe intracellular delivery of a broad range of biological agents including peptides and nucleic acids [84]. As such, design of novel NPs and MPs is being heavily investigated to specifically alter aberrant immune cell behavior to treat

various immune-dysregulated maladies [82–84]. Unsurprisingly, researchers have begun investigating the use of biomaterial-based systems to treat RA, given its pathogenesis being highly immune cell-driven [81]. In addition to this, in order to meet the nutrient and oxygen demands of the infiltrating immune cells, the inflamed synovium becomes a site of angiogenesis and vascular remodeling [105], which under the context of inflammation results in loose endothelial cell junctions and leaky vasculature [106]. As such, RA is a disease that benefits from the EPR effect, allowing nano-sized particles intrinsic ability to preferentially extravasate and accumulate within the joint synovium [107]. Below we will discuss novel biomaterial-based interventions for RA that are currently under investigation, organized based on immune cell target, which are concisely summarized in Table 1. Our discussion spans the fabrication, characterization, and composition of these biomaterial-based therapies, as well as their respective indicated mechanisms of RA interruption.

## Targeting innate immune cells for modulation of rheumatic pathology

### Neutrophils

Neutrophil migration into the inflamed joint exacerbates and sustains joint pathology through cytokine production, NET extrusion, and MMP secretion [52, 108]. To reduce pathological neutrophil activity, Zhang et al. [109] fabricated doxorubicin-conjugated, albumin NPs that incorporated pH-sensitive bonds, allowing doxorubicin liberation upon neutrophil engulfment. This resulted in doxorubicin-mediated neutrophil apoptosis, curbing neutrophil-mediated inflammation in murine models of sepsis and cerebral ischemia/reperfusion [109]. Depletion of neutrophils using cytotoxic NPs may interrupt chronic joint inflammation and protect against neutrophil-mediated degradation of cartilage. Neutrophils are the most abundant circulating cells and possess inherent ability to rapidly migrate to inflamed regions [110]. Harnessing these unique aspects of neutrophil biology, Lyu et al. [111] fabricated human serum albumin NPs (HSA-NPs) decorated with a mannose ligand to provide neutrophil targeting via ligation to neutrophil surface mannose receptors (MRs). This group postulated that NPs can hitch a ride on a synovium-bound neutrophil, allowing for subsequent accumulation of the payload within the inflamed joint [111]. Using a rat model of CIA, Lyu and the group encapsulated methotrexate within the mannose-decorated HSA-NPs and, upon i.v. administration, showed that there was an increase in uptake within neutrophils as well as preferential accumulation within arthritic limbs, compared to the same NP lacking a mannosylated surface [111]. This neutrophil-mediated delivery of a methotrexate-encapsulated NP resulted in significantly

greater alleviation of inflammatory arthritis pathology when compared to unencapsulated methotrexate, and even restoration of bone structure to that of a healthy rat [111]. In similar design, Hu et al. [112] also sought to borrow the neutrophil's innate ability for migration into inflamed tissue. The group fabricated a liposome decorated with sialic acid and encapsulated dexamethasone palmitate in its core [112]. Neutrophils express high levels of the sialic acid receptor, CD62L (also known as L-selectin), which is the receptor that mediates neutrophil slow rolling and subsequent transendothelial migration [113]. The group demonstrated in a rat model of adjuvant-induced inflammatory arthritis that i.v. administration of this sialic acid-conjugated NP laden with dexamethasone palmitate was able to preferentially accumulate within the arthritic limbs, and significantly reduce serum levels of IL-1 $\beta$  and TNF- $\alpha$ , and alleviate joint histopathology, when compared to the vehicle control [112]. Both groups successfully showed that neutrophil-targeting mechanisms could be used to transform the neutrophil into a Trojan horse to efficiently deliver anti-rheumatic drugs directly into the inflamed synovium. Zhang et al. [114] devised another clever strategy to harness the innate attributes of neutrophils for alleviation of synovial inflammation. This team isolated the plasma membrane from activated human neutrophils and used them to coat poly(lactic acid-co-glycolic acid) (PLGA) NPs [114]. These PLGA NPs effectively masqueraded as activated neutrophils and acted as cytokine sinks reducing serum levels of IL-1 $\beta$  and TNF- $\alpha$  [114]. In using this neutrophil-like NP, the group demonstrated a significant reduction in synovial chondrocyte activation in the CIA mouse model, as well as a transgenic humanized TNF- $\alpha$  mouse model of inflammatory arthritis [114]. The overall strategies described here aim at selective apoptosis of neutrophils, or exploiting neutrophil migration, to aid in cargo delivery to the inflamed joint.

### Macrophages

Stymieing the contribution of macrophages to RA pathogenesis is another promising avenue of intervention, due to the central role they play in causing and sustaining joint pathology via cytokine production and participation in osteoclastogenesis [44]. Activated macrophages have been shown to upregulate expression of the folate receptor  $\beta$  (FR $\beta$ ) [115], which is more sparse on non-activated macrophages [116]. Therefore, macrophages expressing FR $\beta$  can preferentially identify inflammatory macrophages and, thus, have amassed keen interest as a selective therapeutic target for treating RA [115]. To this end, ligation of folate to the surface membrane of a biomaterial-based carrier has emerged as an effective strategy for incorporating a targeting component specific for pro-inflammatory macrophages. Verma et al. [117] engineered a double-layered liposome, where the inner liposome encapsulated both prednisolone



**Table 1** Novel biomaterial-based strategies for treating rheumatoid arthritis discussed

Biomaterial category	Constituents	Design	Therapeutic action	Reference
Nanoparticle	Doxorubicin conjugated to bovine serum albumin via a hydrazone bond	Acidic pH-sensitive hydrazone bonds allow liposomes to release doxorubicin upon uptake by neutrophils	Neutrophil apoptosis	[109]
Nanoparticle	Human serum albumin conjugated with mannose receptor	Mannose conjugated to surface targets neutrophil mannose receptors, allowing release of methotrexate cargo in neutrophil-dense sites	Articular immunosuppression	[111]
Liposome	Hydrogenated soy phosphatidylcholine; cholesterol; sialic acid	Sialic acid conjugated to surface targets neutrophil CD62L receptors, allowing release of dexamethasone palmitate in neutrophil-dense sites	Articular immunosuppression	[112]
Nanoparticle	PLGA, neutrophil membranes	Plasma membranes from activated neutrophils were isolated and used to coat a PLGA nanoparticle, allowing sequestration of neutrophil-targeting cytokines	Suppress levels of neutrophil-associated cytokines in the serum	[114]
Liposome	DSPE, DSPC, cholesterol, stearyl amine, folate-PEG-1	Folate conjugation to membrane surface targets activated macrophage folate receptor $\beta$ , allowing release of prednisolone and methotrexate in macrophage-dense sites	Articular immunosuppression and inflammatory macrophage apoptosis	[117]
Liposome/nanoparticle hybrid	DSPC, DSPE-PEG2000, DSPE-PEG2000-folate, cholesterol, calcium phosphate	Folate conjugation to liposome membrane surface targets activated macrophage folate receptor $\beta$ , allowing release of methotrexate and NP containing NF- $\kappa$ B siRNA in macrophage-dense sites	Articular immunosuppression and inflammatory macrophage apoptosis	[119]
Liposome	POPC, NHS, folate-PEG100, monostearate, cholesterol	Folate conjugation to membrane surface and encapsulated catalase targets activated macrophage folate receptor $\beta$ and intracellular ROS, allowing release of methotrexate in macrophage-dense sites	Articular immunosuppression and inflammatory macrophage apoptosis	[120]
Nanoparticle	$\alpha$ -Cyclodextrin, EDC, HPAP, DMAP, CDI, Pluronic F127, DSPE-PEG, DSPE-PEG-FA	Folate conjugation to membrane surface and ROS-reactive bonds targets activated macrophage folate receptor $\beta$ and intracellular ROS, allowing release of dexamethasone in macrophage-dense sites	Articular immunosuppression and inflammatory macrophage apoptosis	[121]
Nanoparticle	Retinal conjugated to galactosyl-dextran via hydrazone bond	Galactose conjugated to membrane surface targets macrophage galactose receptors, hydrazone bonds confer pH-sensitivity, allowing intracellular release of triptolide and all-trans-retinal to target nuclear retinoic acid receptors	Articular immunosuppression and polarize macrophages towards an anti-inflammatory phenotype	[123]
Nanoparticle	FA, PEG-NH <sub>2</sub> , AgNO <sub>3</sub> , NaBH <sub>4</sub>	Folate conjugation to membrane surface of silver NP targets activated macrophage folate receptor $\beta$	Articular immunosuppression and induce macrophage apoptosis or polarize towards an anti-inflammatory phenotype	[125]
Microparticle	PLGA	GM-CSF and TGF- $\beta$ chemoattracts local DCs to an immunosuppressive injection site, allowing release of collagen II and vitamin D3 to promote a tolerogenic dendritic cell phenotype presenting collagen II peptide	Chemoattract and immunomodulate dendritic cells towards tolerance of collagen II	[82]

Table 1 (continued)

Biomaterial category	Constituents	Design	Therapeutic action	Reference
Liposome	L- $\alpha$ -Egg phosphatidylcholine, L- $\alpha$ -egg phosphatidylglycerol	Encapsulation of vitamin D3 alongside an autoantigen promotes a tolerogenic dendritic cell phenotype presenting the autoantigen	Immunomodulate dendritic cells towards antigen-specific tolerance	[138]
Liposome	L- $\alpha$ -Egg phosphatidylcholine	Co-encapsulation of an NF- $\kappa$ B inhibitor alongside an autoantigen promotes a tolerogenic dendritic cell phenotype presenting the autoantigen	Immunomodulate dendritic cells towards antigen-specific tolerance	[140]
Nanoparticle	PEG-b-poly(L-lysine)-b-poly(L-leucine)	Encapsulated c-Rel siRNA allows safe delivery to the joint	Inhibit cytokine production of intra-articular Th1 and Th17 cells	[143]
Liposome/nanoparticle hybrid	DSPC, HAuCl <sub>4</sub>	Encapsulated CoQ10 inhibits transcription factor STAT3, which is important for Th17 activation	Inhibit intra-articular Th17 activation	[146]
Nanoparticle	PLGA	Slow release of encapsulated CCL22 chemoattracts Treg cells to site of injection	Site-specific immunosuppression via migration of Treg cells to site of injection	[148]
Liposome/nanoparticle hybrid	DSPE-PEG-maleimide, cholesterol, $\alpha$ -phosphatidylcholine, Au	Anti-IL-23 antibodies conjugated to surface membrane binds and sequesters IL-23	Intra-articular sequestration of IL-23 suppresses Th17 function	[149]
Nanoparticle	PEG-PLGA	Encapsulated BAFF siRNA allows safe delivery to the joint	Intra-articular downregulation of BAFF, resulting in B cell apoptosis	[156]
Nanoparticle	PLGA	Fibrin peptide conjugated to membrane surface targets autoreactive B cells, encapsulated HIV-1 peptide induces cytotoxic death	Antigen-specific cytotoxicity of autoreactive B cells	[157]
Liposome	PEG-DSPE, DSPC, cholesterol	Cyclic citrullinated peptide conjugated to membrane surface targets autoreactive B cells, and glycan conjugated to membrane surface targets the inhibitory B cell receptor CD22	Antigen-specific inhibition of autoreactive B cells	[158]
Liposome	DSPC, cholesterol, mannose	Mannose conjugated to the surface targets mannose receptors of macrophages undergoing osteoclastogenesis, allowing for release of p-coumaric acid	Prevent articular macrophages from undergoing osteoclastogenesis	[163]
Liposome	Berberine, DSPC, mannose	Mannose conjugated to the surface targets mannose receptors of macrophages undergoing osteoclastogenesis, berberine conjugated to the surface induces upregulation of anti-inflammatory miR-23a	Prevent articular macrophages from undergoing osteoclastogenesis	[164]
Nanoparticle	PLGA	Encapsulated indocyanine green and perfluorocarbon allows photodynamic and sonodynamic targeting of fibroblasts	Cytotoxic death of fibroblast-like synoviocytes	[171]
Hydrogel	Tetrazine-HA, trans-cyclooctene-HA, DMTMM	Encapsulated methotrexate within a cross-linked hyaluronic acid depot	Articular immunosuppression via localized sustained release of hyaluronic acid and methotrexate	[178]
Nanoparticle hydrogel	Soluplus, Kolliphor P407	Thermosensitive NPs encapsulating tacrolimus induce cross-linking upon exposure to body temperature	Articular inhibition of T cell activation via localized sustained release of tacrolimus	[179]

Table 1 (continued)

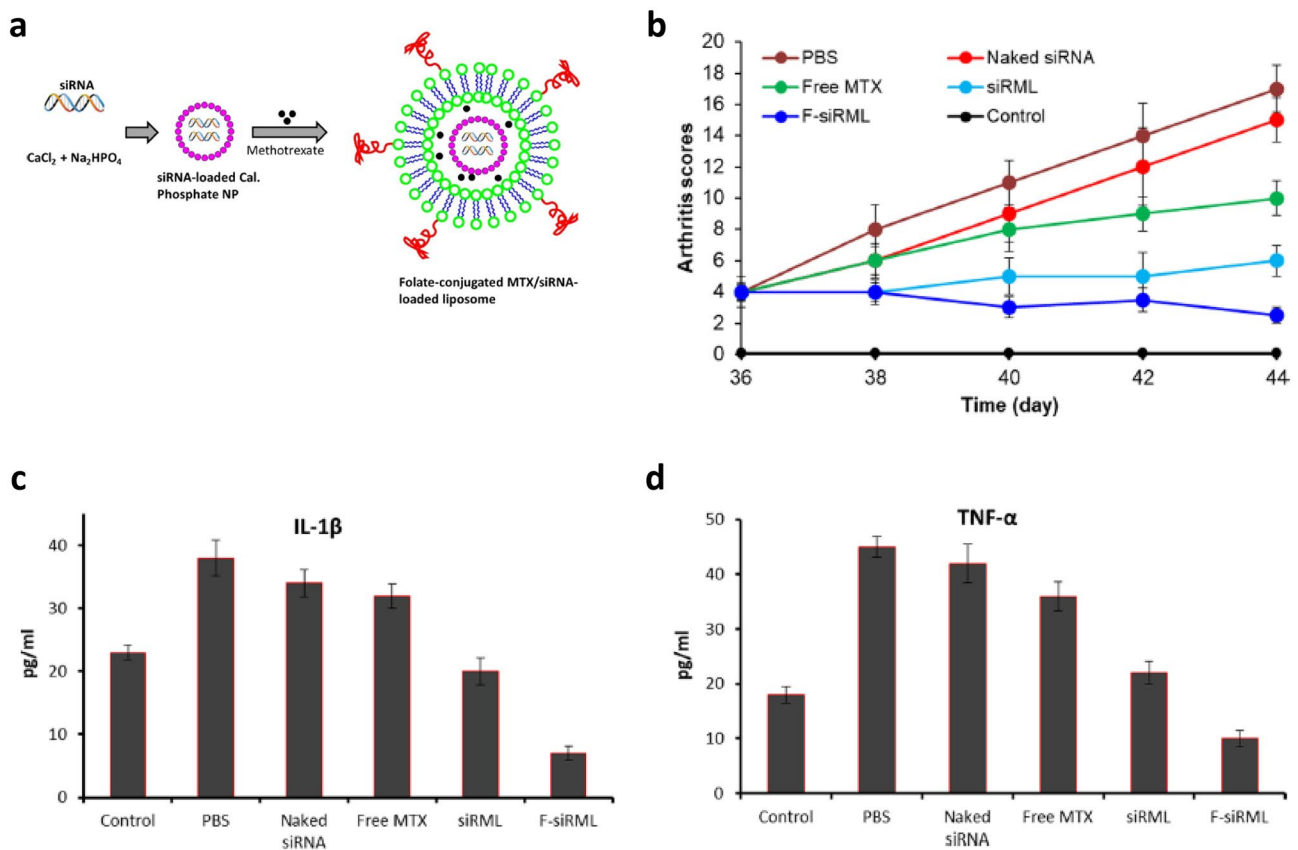
Biomaterial category	Constituents	Design	Therapeutic action	Reference
Hydrogel	Pluronic F127, HA, PGA	Hyaluronic acid hydrogel encapsulating infliximab	Articular immunosuppression and reduction in levels of TNF- $\alpha$ via localized sustained release of hyaluronic acid and infliximab	[180]
Scaffold and hydrogel	HA-hydrazide, HA-aldehyde, sodium hyaluronate, EDC	Scaffold seeded with adipose-derived mesenchymal stem cells combined with an infliximab-encapsulated hydrogel	Reduction in levels of articular TNF- $\alpha$ via localized sustained release of infliximab, also promotes cartilage and bone repair	[181]
Hydrogel	Disulfide-cross-linked polyethyleneimine, Pluronic F127, F68	Indomethacin, methotrexate, and MMP-9 siRNA were encapsulated within a hydrogel	Articular immunosuppression and inhibition of cartilage degradation via localized concentration and sustained release of indomethacin, methotrexate, and MMP-9 siRNA	[182]

*DSPE* 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, *DSPC* 1,2-distearoyl-sn-glycero-3-phosphocholine, *PEG* polyethylene glycol, *EDC* 1-ethyl-3-(3-dimethylamino)propyl carbodiimide hydrochloride, *NHS* N-hydroxysuccinimide, *POPC* 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, *HPAP* 4-(hydroxymethyl)phenylboronic acid pinacol ester, *DMAP* 4-dimethylaminopyridine, *CDI* 1,1'-carbonyldiimidazole, *FA* folic acid, *PLGA* poly(lactic acid-co-glycolic acid), *HA* hyaluronic acid, *DMTMM* 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride, *PGA* poly( $\gamma$ -glutamic acid)

and methotrexate, while the outer membrane was decorated with folate [117]. Following i.v. administration of this double liposome into the CIA rat model, the group observed preferential accumulation of both prednisolone and methotrexate within the inflamed joint, compared to the non-inflamed joint [117]. The transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) is a master regulator of a pro-inflammatory response in macrophages [118]. Duan and Li [119] used folate-functionalized liposomes to co-deliver methotrexate alongside a NP containing small interfering RNA (siRNA) against NF- $\kappa$ B (Fig. 3a) into the diseased joint. They hypothesized that macrophage uptake of this liposome would result in NF- $\kappa$ B silencing, impairing activation and production of pathogenic cytokines. The group performed i.v. administration of this liposome into the CIA mouse model and observed amelioration of overt inflammatory arthritis symptoms (Fig. 3b), as well as a fourfold decrease in serum levels of IL-1 $\beta$  (Fig. 3c) and a twofold decrease in TNF- $\alpha$  (Fig. 3d), compared to the same liposome without folate conjugation [119], demonstrating the effectiveness of the folate targeting system. In efforts to further refine a delivery system that excels at preferentially targeting activated macrophages, Chen et al. [120] incorporated a catalase within the folate-conjugated liposome. In addition to upregulation of FR $\beta$ , activated macrophages also express high levels of intracellular reactive oxygen species (ROS) in the form of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [120]. As such, Chen and co-authors took advantage of this secondary characteristic of activated macrophages by adding catalase into their delivery system. Once the liposome is engulfed by the macrophage, H<sub>2</sub>O<sub>2</sub> diffuses into the liposomal core and is exposed to catalase, breaking it down into H<sub>2</sub>O and O<sub>2</sub>, which leads to destabilization of the liposome and rapid release of the methotrexate payload [120]. Incorporation of two targeting components further enhances the rigor in selectively targeting only the inflammatory macrophages, and contributes to mitigating off-target effects such as regulatory macrophages. In vitro studies showed that in ROS-abundant conditions, encapsulation of catalase improved the cumulative release of methotrexate from the liposome by a fourfold increase, compared to the same liposome without catalase [120]. Additionally, i.v. delivery of this catalase- and folate-functionalized liposome laden with methotrexate into CIA mice exhibited greater inflammatory arthritis disease remission, compared to the folate-conjugated liposome containing methotrexate only, demonstrating that incorporation of catalase improves the efficacy of this carrier system in treating inflammatory arthritis [120]. In a similar fashion, Ni et al. [121] formulated a folate-conjugated NP out of 4-phenylboronic acid pinacol ester-conjugated  $\alpha$ -cyclodextran (Oxi- $\alpha$ CD), another strategy to incorporate a ROS-sensitive component

into the carrier system, and they subsequently encapsulated dexamethasone into its core [121]. This also yielded a ROS-reactive nanocarrier armed with two attributes that target inflammatory macrophages [121]. Administration of this NP into the CIA mouse model resulted in accumulation within the inflamed joint and downregulation of TNF- $\alpha$  and B cell-activating factor (BAFF), and alleviated inflammatory arthritis symptoms within the paw [121]. Ligation of macrophage nuclear retinoic acid receptors (RARs) to its ligand, all-trans-retinal, promotes polarization from an inflammatory M1 phenotype towards a regulatory M2 phenotype [122]. In efforts to re-educate pro-inflammatory synovial macrophages, Li et al. [123] incorporated three components into a pH-sensitive NP, (1) all-trans-retinal, (2) galactose, and (3) triptolide, an anti-inflammatory compound, into a dextran NP containing hydrazone bonds [123]. This NP served to target macrophages via the galactose-type lectin (MGL) receptor, and subsequent intracellular delivery of all-trans-retinal and triptolide would coerce polarization towards a regulatory

phenotype [123]. Administration of this NP into CIA mice achieved a reduction of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  within the synovium [123]. On the topic of re-polarization, silver NPs have been shown to increase intracellular Ag<sup>+</sup>, promoting macrophage polarization towards an M2 phenotype through inhibition of phosphoinositide 3-kinase (PI3K)/Akt signaling, decreasing expression of glucose transporter expression, and inducing autophagy [124]. In a similar macrophage re-educating strategy, Yang et al. [125] functionalized a silver NP with folate and successfully induced the death of inflammatory macrophages, or their polarization towards an M2 phenotype in the CIA murine model [125]. Taken altogether, folate-functionalized nanoparticles provide an effective and customizable framework for targeting problematic macrophages. The common and effective strategies employed were either depletion of inflammatory macrophages or re-polarization towards a regulatory phenotype, which were both effective in alleviating inflammatory arthritis symptoms.



**Fig. 3** In vivo targeting of macrophages using a folate-conjugated liposome/nanoparticle hybrid encapsulating NF- $\kappa$ B siRNA and methotrexate. **a** Schematic diagram of the liposome/nanoparticle hybrid carrier. **b** Folate-functionalized carrier encapsulating NF- $\kappa$ B siRNA and methotrexate was the most efficacious at reducing the arthritis score in the collagen-induced arthritis (CIA) mouse. **c** Forty-four

days post-treatment of a CIA mouse, the folate-functionalized carrier encapsulating NF- $\kappa$ B siRNA and methotrexate reduced serum levels of IL-1 $\beta$  fourfold and **d** TNF- $\alpha$  by twofold compared to the same carrier lacking folate-functionalization. Adapted from Duan and Li [119]. Open access

## Dendritic cells

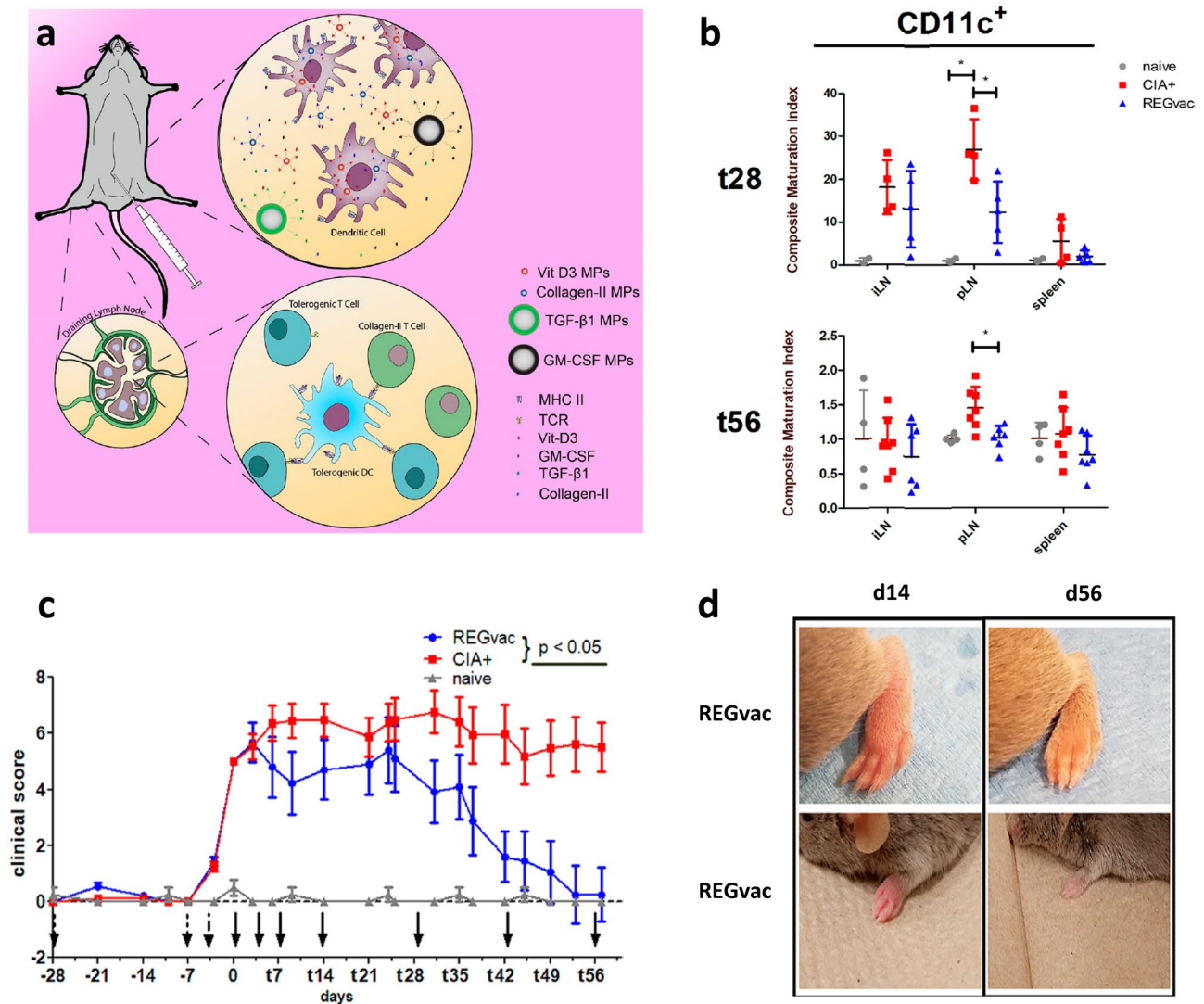
Dendritic cells play significant and opposing roles in promoting autoimmunity [126] or maintaining tolerance [127]. Upon exposure to pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), DCs undergo maturation and upregulate inflammatory cytokines IL-12 and IFN- $\alpha$ , as well as co-stimulatory molecules CD80 and CD86, ultimately orchestrating an inflammatory immune response against the associated pathogen [128, 129]. On the other hand, DCs exposed to immunosuppressive molecules such as IL-10 and TGF- $\beta$ 1 induce tolerogenic dendritic cells (tDCs) which express low levels of IL-12, IFN- $\alpha$ , CD80, and CD86 [129]. Tolerogenic DCs are proficient mediators of tolerance, the mechanism of which is primarily through inducing anergy of autoreactive T cells and induction of regulatory T cells (Tregs) [127, 129, 130]. As such, modulating DCs towards a tolerogenic phenotype has amassed considerable interest for their potential in restoring tolerance in autoimmune diseases [83, 131, 132], including transplant rejection [133], type I diabetes [130, 134], multiple sclerosis [135], and RA [82]. A plethora of biomolecules have been identified that promote induction of tDCs such as  $1\alpha,25$ -dihydroxycholecalciferol (vitamin D3), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), IL-10, galectin-1, and glucocorticoids, among others [129]. Of particular interest, vitamin D3 has been extensively studied for its proficiency in DC modulation that is permissive of downstream induction of Treg cells [130, 136]. Autologous delivery of tDCs directly into the arthritic joint has undergone a phase I clinical trial with promising results [137] (NCT01352858, NCT03337165). To harness endogenous tDC mechanisms, Allen et al. [82] devised a novel dual PLGA microparticle system termed “REGvac” to induce generation of tDCs in vivo [82]. This microparticle system consists of (i) a 1- $\mu$ m phagocytosable microparticle encapsulating type II collagen and vitamin D3 to facilitate uptake by dendritic cells and (ii) a 30- $\mu$ m unphagocytosable microparticle encapsulating GM-CSF and TGF- $\beta$ 1, to mediate DC chemotaxis into a pro-tolerogenic environment (Fig. 4a) [82]. Subcutaneous delivery of REGvac in the CIA mouse prevented DC maturation (Fig. 4b) ameliorated inflammatory arthritis symptoms (Fig. 4c and d), improved gait, and induced Treg cells within the draining lymph nodes of the inflamed joint [82]. In a similar vein, Galea et al. [138] formulated a liposome encapsulating vitamin D3 (calcitriol) alongside the model antigen ovalbumin (OVA) to investigate the capacity for inducing antigen-specific tolerance [138]. Using the TCR-transgenic OVA-specific T cell line DO11.10, subcutaneous immunization with this vitamin D3/OVA liposome was found to induce expansion of OVA-specific Tregs and induce anergy of OVA-specific non-Treg T cells via upregulation of PD-1 [138]. Under inflammatory conditions where mice were primed with OVA and the

adjuvant QuilA, the calcitriol/OVA liposome induced DC downregulation of MHC class II, and upregulated expression of PD-L1, which was found to suppress OVA-specific T cell expansion and induce Tregs in a PD-L1-dependent manner [138]. Next, Galea and co-authors assessed the capability of this antigen/calcitriol liposome for inducing tolerance in a mouse model of proteoglycan-induced inflammatory arthritis, and also type IV collagen  $\alpha$ 3-induced model of vasculitis, where the group observed suppression of arthritis score and also a reduction in antigen-specific CD4<sup>+</sup> T cells [138]. Inhibition of the transcription factor NF- $\kappa$ B has been shown to impair DC maturation [139]. Capini et al. [140] showed that DC-targeted NF- $\kappa$ B inhibition alongside autoantigen delivery was able to induce antigen-specific FoxP3<sup>+</sup> Treg-mediated suppression of inflammatory arthritis using an antigen-induced arthritis (AIA) mouse model, with methylated BSA (mBSA) as the antigen [140]. In order to achieve this, the group fabricated egg phosphatidylcholine liposomes encapsulating one of three different NF- $\kappa$ B inhibitors: curcumin, quercetin, or Bay 11-7082, as well as mBSA in order to facilitate direct co-delivery of the NF- $\kappa$ B inhibitor and mBSA directly into the cytosol of DCs via subcutaneous injection into the mouse tail [140]. Following these promising results, Benham et al. [141] conducted a phase I clinical trial using autologous DCs treated with Bay 11-7082 and exposed to four different RA-associated citrullinated antigens (termed Rheumavax) to treat RA patients. A single dose of Rheumavax was administered intradermally into the upper thigh and yielded no or minimal adverse effects and was able to reduce RA disease activity [141]. Taken together, the potential for using biomaterial-based strategies to immunoengineer DCs for antigen-specific tolerance induction in RA is robust. Targeting DCs in RA therapy is a sound enterprise given its role as a nucleating factor in the induction of ACPAs [5].

## Biomaterial-based instruction of adaptive immune elements for correction of RA

### T cells

The transcription factor c-Rel is a master regulator for inflammatory cytokine production in Th1 and Th17 cellular responses and has been implicated in various autoimmune diseases [142]. Fan et al. [143] used poly(ethylene glycol)-b-poly(L-lysine)-b-poly(L-leucine) (PEG-PLL-PLLeu) triblock polymers to construct a nanoparticle encapsulating siRNA against c-Rel transcripts [143]. Delivery of the c-Rel-loaded NPs into CIA mice halted disease progression and ameliorated joint pathology [143]. In vivo delivery of c-Rel siRNA NP exhibited a reduction in DC ability to activate T cells, resulting in abrogation of Th1- and Th17-associated cytokines, IFN- $\gamma$  and IL-17, as well as a



**Fig. 4** In vivo immunomodulation of dendritic cells using a dual microparticle delivery system in the collagen-induced arthritis (CIA) mouse model. **a** Schematic of constituents of the microparticle system (REGvac) and proposed cellular targets. REGvac was delivered subcutaneously in the abdominal region, draining to the inguinal lymph nodes. **b** Twenty-eight days and 56 days post-treatment,

spleen, inguinal, and popliteal lymph nodes were harvested and flow cytometry was performed to measure dendritic cell maturation status. **c** CIA mice were clinically scored for limb erythema and swelling. **d** Representative photographs of the paws of a REGvac-treated CIA mouse at 14 days and 56 days post-treatment. Adapted from Allen et al. with permission [82]

reduction in inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, and IL-23 [143]. Additionally, the c-Rel siRNA NP reduced T cell infiltration into the diseased joint [143]. The antioxidant coenzyme Q10 (CoQ10) inhibits the transcription factor STAT3, a critical regulator for Th17 effector function [144, 145]. Jhun et al. [146] used a liposome and gold hybrid nanoparticle to encapsulate CoQ10 (LGNP-CoQ10) for curbing Th17 activity in the CIA mouse model [146]. These CoQ10 NPs were administered orally, and the authors found a greater reduction in arthritic score, and a reduction in Th17 numbers and cytokine levels, compared to unencapsulated CoQ10 [146]. Regulatory T cells are

remarkable mediators of tolerance through production of IL-10 and their ability to competitively inhibit DC-mediated co-stimulatory activation of T cells [28, 126]. Moreover, the absence of Treg cells results in spontaneous autoimmunity [147]. As such, engineered biomaterial strategies to enhance Treg function is an enticing enterprise. Notably, Fisher et al. [148] encapsulated the Treg chemokine, CCL22, within a PLGA NP. Administration of these CCL22 NPs into an allografted rat hind limb resulted in local recruitment of Tregs and mediated long-term graft acceptance [148]. This study provides an interesting concept of exploiting Treg recruitment to a target site that may be applied to rheumatic

joints. Activated macrophages and dendritic cells secrete high levels of IL-23 to sustain Th17 activity [46, 47]. Th17 cells produce RANKL themselves, but also produce IL-17 and IL-22 to induce RANKL expression in synoviocytes, ultimately resulting in osteoclast activation [46, 47]. Rather than systemic administration of neutralizing antibodies, Lima et al. [149] fabricated a liposome functionalized with anti-IL-23 neutralizing antibodies to concentrate the site of cytokine neutralization within the diseased joint. The authors tested the anti-IL-23 liposomes on the serum of RA patients and were able to achieve a significant reduction of IL-17 production [149], indicating that IL-23 neutralization successfully impaired Th17 cell ability to make IL-17. Functionalizing particles with antibodies to neutralize cytokines is a promising strategy, as there are many different cytokines within the arthritic joint that sustain inflammation. In addition to cytokines, this strategy may also be employed to neutralize harmful autoantibodies within circulation [150], and given how RA is an autoantibody-driven disease [35], an engineered particle that reduces the serum levels of ACPAs and RF may be an effective therapeutic venture for treating RA.

### B cells

As the culprits responsible for maturing and generating ACPAs, B cells undeniably play a destructive role in RA pathogenesis and in sustaining chronic disease [27, 33, 34, 151, 152]. As such, depletion of B cells in RA has seen therapeutic effects [65, 153]. The pro-survival factor, BAFF, is produced by APCs and is important for B cell development and maturation [154], and also aids in T cell co-stimulation [155]. Additionally, BAFF overexpression has been linked to autoimmunity [154]. In order to curb RA-associated pathogenic B cell activity, Wu et al. [156] constructed a PEGylated PLGA nanoparticle encapsulating siRNA against BAFF. Administration of this NP i.v. into CIA mice resulted in amelioration of paw pathology, a reduction in circulating anti-collagen antibodies, and a reduction in the number of B cells [156]. In an effort to introduce a targeting component specific for only autoreactive B cells, Pozsgay et al. [157] covalently fused a fibrin peptide, bindable by ACPAs, and a peptide from human immunodeficiency virus type 1 (HIV-1), to the surface of PLGA NPs, termed “bifunctional NPs.” The fibrin peptide provides specificity for ACPA-producing B cells, and the HIV-1 peptide promotes complement-mediated cytotoxicity [157]. B cells were isolated from RA patients and, upon Toll-like receptor 7/8 stimulation, were found to secrete antibodies against the fibrin peptide [157]. Following this, the bifunctional NP was introduced in vitro with the RA-derived B cells, alongside human sera to provide complement components [157]. The authors observed a reduction in fibrin-specific B cells in the presence of both bifunctional NPs and

human sera, but not in heat-inactivated human sera [157], suggesting that the bifunctional NPs were indeed inducing complement-mediated death of fibrin-specific B cells. In a similar precision-based design that targets autoreactive B cells, Bednar et al. [158] fabricated a liposome conjugated with (1) a high-affinity glycan ligand for the inhibitory B cell receptor, CD22, and (2) a cyclic citrullinated peptide, to facilitate interaction with B cells specific for this autoantigen. Upon B cells encountering this dual-functionalized liposome, the glycan ligand engages CD22 and induces B cell deletion, subsequently restoring B cell tolerance towards the citrullinated antigen [158]. Bednar and co-authors demonstrated that this liposome was able to induce tolerance in autoreactive B cells isolated from RA patients, as well as in SJL/J mice immunized against cyclic citrullinated peptides [158]. Here, the significance of applying biomaterial-based designs in B cell-centric therapies is enabling the precise targeting of autoreactive B cells, thereby minimizing off-target effects and decreasing toxicities.

### Using particles for manipulation of RA-promoting, non-immune cells

In RA, osteoclast generation is highly upregulated and they are the primary cells that facilitate bone damage [159]. Osteoclastogenesis is a process by which RANKL-activated macrophages undergo cell fusion to yield a multinucleated osteoclast [160]. It has been reported that mononuclear precursors gradually crescendo surface expression of mannose and MRs during the process of osteoclastogenesis, and subsequent MR ligation by mannose is important for mediating cellular fusion [161]. Neog et al. [162] have previously demonstrated that the polyphenol p-coumaric acid exerts anti-inflammatory and anti-osteoclastogenic effects in an AIA rat model. In a follow-up study, Neog and co-authors [163] took advantage of osteoclast upregulation of MR and incorporated mannose into the liposomal surface to provide a p-coumaric acid-encapsulated liposome with an osteoclast-targeting component [163]. In the AIA rat model, biodistribution of this mannosylated liposome was demonstrated to preferentially accumulate in the arthritic paw following i.v. administration [163], verifying the efficacy of mannose to guide liposomes to the inflamed paw. Subsequent isolation of primary synovial macrophages from the AIA rat treated with the p-coumaric acid-laden liposome showed impaired ability to perform osteoclastogenesis and bone resorption, compared to methotrexate-treated and untreated synovial macrophages [163]. In another follow-up study from the same group, Sujitha et al. [164] incorporated berberine into the surface of the mannosylated liposome, which has been previously shown to induce silencing of inflammatory genes in macrophages through upregulation of microRNA (miRNA)-23a [165]. Using bone-marrow derived macrophages, the

berberine-loaded mannosylated liposome demonstrated *in vitro* inhibition of osteoclastogenesis and impaired bone resorption ability in existing osteoclasts [164]. Furthermore, various gold nanoparticle formulations have been shown to inhibit osteoclastogenesis and bone resorption [166–169], likely through exerting intracellular antioxidant effects [166]. Fibroblast-like synoviocytes (FLSs) play key roles within the inflamed joints. In RA, articular inflammation activates FLSs to invade the cartilage space, participate in extracellular matrix (ECM) remodeling via MMP production, and also produce inflammatory cytokines such as IL-6, GM-CSF, and RANKL [170]. Tang et al. [171] formulated a photosensitive PLGA NP by encapsulating indocyanine green (ICG), as well as liquid perfluorocarbon, as an O<sub>2</sub> carrier to introduce ROS. Tang and co-authors sought to selectively kill FLSs through a combinatorial intervention of photodynamic and sonodynamic therapy. In this system, introduction of near-infrared (IR) energy followed by ultrasonic irritation allows targetable and triggerable ROS-mediated cytotoxicity within a target site [171]. Tang and co-authors utilized the synoviocyte cell line, MHA7, and demonstrated that *in vitro*, their photosensitive NP was able to induce robust ROS-mediated death of synoviocytes upon exposure to near-IR and ultrasonic energies [171].

### Scaffolds help to build therapy

To this point, our discussion has centered around particulate formulations largely because their unique properties allow researchers to effectively target immune cells. However, bulkier material constructs such as scaffolds and hydrogels are also under intense investigation for applications in therapy of rheumatic disease. Biomaterial-based scaffolds and hydrogels offer numerous benefits for tissue engineering, drug delivery [172], cancer therapy [173], and immunomodulatory applications [174]. Engineered constructs possess a myriad of customizable attributes such as encapsulation, type of polymer, firmness, size of pores, and release kinetics [172]. These biocompatible constructs excel as support depots through localized sustained release of bioactive molecules [172]. They can also be seeded with autologous precursor cells to promote *in situ* growth of new organs or tissue [175], and can also be used to deliver regenerative cells alongside growth factors and hormones to promote cell proliferation and tissue repair at the target site [176]. In terms of immunomodulatory applications, scaffolds and hydrogels possess unique prowess at modifying the local environment. Encapsulation of immunomodulatory agents within engineered constructs allows these bioactive molecules to concentrate at the site of interest and exert potent effects on the nearby immune cells [174]. For the majority of the disease, rheumatoid arthritis pathology is contained to the joints [35]. This specific localization of diseased sites makes the use of intra-articular

implantation of scaffolds and hydrogels an attractive therapeutic strategy. Intra-articular hyaluronic acid (HA) supplementation has been commonly used as an anti-inflammatory agent to treat inflammatory arthritides [177]. Seo et al. [178] incorporated methotrexate into a cross-linked HA depot to promote long-term sustained release within the diseased joint, which helps to mitigate side effects of systemic methotrexate treatment. The group then administered this depot intra-articularly into the CIA rat model and observed sustained retention of elevated HA and methotrexate within the synovium, resulting in alleviation of inflammatory arthritis joint pathology, improved articular cartilage thickness and bone formation, and downregulation of TNF- $\alpha$  and IL-6 [178]. Wu et al. [179] sought to devise a self-assembling hydrogel system by using NPs made of caprolactam–polyvinyl acetate–polyethylene glycol (Soluplus). The Soluplus NPs are thermosensitive, thickening in viscosity at body temperature, allowing for hydrogel formation upon injection into the target site [179]. Wu and co-authors encapsulated tacrolimus, an inhibitor of T cell activation, within the Soluplus NPs, allowing for a self-assembling drug depot upon injection [179]. The group administered the tacrolimus-laden Soluplus NPs via subcutaneous injection adjacent to the arthritic joints, and observed a significant reduction in edema within the arthritic paw over time, compared to the same hydrogel without tacrolimus [179]. Chen et al. [180] formulated a hydrogel composed of Pluronic F127, poly( $\gamma$ -glutamic acid), and hyaluronic acid, and encapsulated infliximab, an anti-TNF- $\alpha$  antibody. Through incorporation of infliximab into the hydrogel, Chen and co-authors aim to sequester infliximab within the diseased joint, thereby mitigating systemic suppression of TNF- $\alpha$  [180]. In a rabbit model of ovalbumin (OVA)–induced RA, the group injected the hydrogel intra-articularly 1 week post RA induction. After 6 weeks, they observed alleviation of joint pathology and cartilage destruction, as well as a reduction in synovial fluid levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-17, compared to the same hydrogel without infliximab [180]. Zhao and co-authors [181] sought to add a healing component to their strategy in addition to alleviation of joint pathology. The group utilized an infliximab-based hydrogel combined with a 3D-printed porous metal scaffold (3DMS) that was seeded with adipose-derived mesenchymal stem cells (ADSCs), where introduction of the ADSCs would aid in the rebuilding of the articular ECM [180]. This scaffold was implanted intra-articularly within the OVA-induced rabbit model and led to amelioration of RA pathology and promoted restoration of cartilage and bone repair [180]. Similarly, Yin et al. [182] prepared a hydrogel system with F127 and a polyethyleneimine NP encapsulating (1) the non-steroidal anti-inflammatory drug, indomethacin; (2) methotrexate; and (3) siRNA against MMP-9, to halt further damage to the articular ECM. Yin and co-authors administered this hydrogel into the inflamed joint of a CIA mouse



and observed significant amelioration of joint pathology as well as a reduction in TNF- $\alpha$ , IL-6, and MMP-9 in serum and in synovial fluid [182]. Remarkably, they also observed joint architecture to return close to baseline [175].

## Future outlooks and conclusion

To date, RA remains a prevalent health burden with no cure and current treatments fail to achieve consistent remission and are limited by toxicities, adverse effects, and, most notably, increased risk of infection [17, 55]. Amid an evolving SARS-CoV-2 pandemic, major concerns regarding the use of general immunosuppressants for autoimmune patients [21, 22] and solid organ transplant recipients [183–185] are in contention. It is a tragic dilemma when the life-saving therapy may possibly escalate the risk of a SARS-CoV-2 infection to a life-threatening event [21, 186], especially when these drug regimens generally require lifelong management [55]. The need to develop autoimmune therapies that sidestep broad immunosuppression has never been greater, and application of biomaterial-based designs (liposomes, nanoparticles, microparticles, hydrogels, or scaffolds) to this dilemma offers remarkable promise. Discovery and incorporation of biomaterials into cutting-edge nanomedicines have profoundly expanded the toolbox of therapeutic strategies, granting access to problem-solving from perspectives previously unavailable. While broad immunosuppressive drugs are invaluable and necessary, there is still copious room for improvement. By way of developing new biomaterial-based therapies, the concept of precision has emerged as potentially the most valuable enterprise. This review has revealed that common themes of biomaterial-inspired therapeutic strategies aim to mitigate systemic effects through tactics such as facilitating drug delivery to the target site, or incorporating a targeting component into the delivery vehicle. In this regard, our current understanding of autoimmunity has highlighted the significance of thwarting RA in a specific manner in order to minimize off-target effects. For example, this can be through specific depletion of a problematic cell type or induction of tolerance towards autoantigens, which permits general immunity to still respond appropriately to pathogens, such as SARS-CoV-2. In particular, inducing antigen-specific tolerance is a highly coveted outcome because it does not immunocompromise the patient, whereas conventional drug therapies render the patient immunocompromised and susceptible to infection [187]. To this end, several biomaterial-inspired strategies are being investigated to restore antigen-specific immunological tolerance towards the autoantigens that propagate RA [82, 138, 140, 157, 158]. Pertaining to RA pathogenesis, this implicates DCs, and autoreactive B and T cells for their pivotal roles in bringing forth ACPAs [24], and as such are promising targets in the pursuit of restoring

tolerance. For example, our understanding of immunology, progression of RA, and biomaterials has provided the groundwork for using nanocarriers to coerce immune cells into restoring immunological tolerance towards autoantigens, as evidenced by the studies carried out by Allen et al. [82], Galea et al. [138], and Capini et al. [140] where induction of antigen-specific tDCs was able to exert rippling downstream therapeutic effects through induction of antigen-specific Treg cells and anergy of autoreactive T cells. Continued exploration of the mechanisms of tolerance induction during the disease state of RA is very welcome. We believe taking an omic approach to advance knowledge would unlock a multitude of new potential targets that we may use biomaterials to investigate. For example, a multi-omic interrogation of an induced tDC from an inflammatory arthritis disease state will inform us how our biomaterial-based strategies are modulating a specific cell type to induce therapeutic effects. Additionally, cross-referencing differentially expressed genes from RNA-seq, with differentially accessible regions from assay for transposase-accessible chromatin (ATAC)-seq, and gene regulatory motifs from chromatin immunoprecipitation (ChIP)-seq will reveal critical up- or downregulated pathways, transcription factors, or genes that will become attractive targets for future biomaterial-based strategies to induce tolerance in an antigen-specific manner, as we believe this venture to be the most groundbreaking one in the pursuit of new biomaterial-based immunotherapies for RA.

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## Declarations

**Consent for publication** The authors give consent for publication.

**Conflict of interest** The authors declare no competing interests.

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