Cationic nanoparticle as an inhibitor of cell-free DNA-induced inflammation

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Cell-free DNA (cfDNA) released from damaged or dead cells can activate DNA sensors that exacerbate the pathogenesis of rheumatoid arthritis (RA). Here we show that ~40 nm cationic nanoparticles (cNP) can scavenge cfDNA derived from RA patients and inhibit the activation of primary synovial fluid monocytes and fibroblast-like synoviocytes. Using clinical scoring, micro-CT images, MRI, and histology, we show that intravenous injection of cNP into a CpG-induced mouse model or collagen-induced arthritis rat model can relieve RA symptoms including ankle and tissue swelling, and bone and cartilage damage. This culminates in the manifestation of partial mobility recovery of the treated rats in a rotational cage test. Mechanistic studies on intracellular trafficking and biodistribution of cNP, as well as measurement of cytokine expression in the joints and cfDNA levels in systemic circulation and inflamed joints also correlate with therapeutic outcomes. This work suggests a new direction of nanomedicine in treating inflammatory diseases.

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Rheumatoid arthritis (RA) afflicts approximately 1% of population worldwide. Its symptoms include swollen and deformed joints due to inflammatory damage of the bone and cartilage. Severe cases could lead to systemic failure including cardiovascular, pulmonary, psychological, and skeletal disorders. Current drug therapy relies on glucocorticoids and non-steroidal anti-inflammatory drugs. Synthetic anti-rheumatic drugs, such as methotrexate show strong side effects like teratogenicity and hepatotoxicity. Biologics such as anti-TNF-α that acts by inhibiting inflammatory functions may cause a high risk of infection, like tuberculosis, due to a weakened immune system. Moreover, biologic drug is too expensive for patients in developing countries.

It is still a great challenge to treat RA, especially for its complicated pathogenesis. Recent studies suggested that cell-free DNA (cfDNA), played a critical role in RA development. The origin of cfDNA comes from degradation of DNA released from apoptotic or dead cells, nuclei expelled from erythroid precursors, mitochondrial DNA, or neutrophil extracellular traps (NETs). Clinical study showed that cfDNA level in serum of patients was elevated. The cfDNA content in the synovial fluid of patients is even thousands of times higher than that in the plasma, serving as a biomarker for RA diagnosis. cfDNA eliciting immunity response in vivo through varied ways has been recognized. The immune complexes formed by cfDNA and autoantibody, as well as proteins like HMG1 and LL37 may be internalized into immune cells and activate the Toll-like receptors (TLR) to secrete inflammatory cytokines to cause tissue inflammation. Furthermore, other studies showed a profound relationship between cfDNA and RA development. When excessive DNA was accumulated in the plasma of nuclease-deficient mice, it would lead to abnormally high-level cytokines that in turn caused joint inflammation and bone destruction. These studies suggest that cfDNA may be a target for RA therapy. Recently, Sullenger et al. demonstrated that cationic polymers can be used to neutralize cfDNA to inhibit inflammation in an acute liver injury model and in alleviating symptoms of systemic lupus erythematosus (SLE).

Hypothesizing that blocking the TLR activation may reduce inflammation in RA, we propose the use of cationic polymers to scavenge damage-associated molecular patterns (DAMP) molecules as a new strategy to treat RA. In addition, we opt to focus on cationic nanoparticles (cNP) instead of soluble polycations because of a potentially higher nucleic acid (NA) scavenging capacity and a more favorable biodistribution in the inflamed joints. In the development of RA, inflammation induces angiogenesis to form a leaky vasculature in the inflamed joints. We show that cNP composed of the diblock copolymer of poly(lactic-co-glycolic acid) (PLGA) and poly(2-(diethylamino)ethyl methacrylate) (PDMA) with a size around 40 nm have a high DNA-binding affinity, which in turn efficiently inhibit primary SFMC and FLS activation by cfDNA from RA patients. Intravenous injection of cNP into a Cpg-induced mouse or collagen-induced arthritis (CIA) rat model would relieve RA symptoms with respect to ankle and tissue swelling and reduce bone and cartilage damage. The cNP are effective in treating the CIA model at both the early stage, as well as the established stage of RA progression. Treatment with cNP also leads to partial mobility recovery in the CIA-induced RA rats. The favorable therapeutic outcome is corroborated with determination of intracellular trafficking, biodistribution, cfDNA levels in systemic circulation and inflamed joints, and cytokine levels in the joints.

**Results**

cNP shows high DNA binding and inhibition of inflammation. cNP ca. 40 nm in diameter were prepared by self-assembly of PLGA-block-PDMA block copolymer, PLGA-b-PDMA463 (see chemical structure in Supplementary Figure 1a). The particle has a structure of PLGA core and cationic PDMA shell. The homopolymer with a similar chain length to the PDMA shell of cNP, PDMA470, was used for comparison. In PBS of pH 7.4, cNP and PDMA are polycationic materials with the zeta potential of +18 and +14 mV, respectively. The binding affinity of the cNP and PDMA with calf thymus DNA was evaluated by competitive binding with ethidium bromide (EtBr) and in the plasma of patients15,16. Furthermore, several other studies showed a profound relationship between cfDNA and RA development. When excessive DNA was accumulated in the plasma of nuclease-deficient mice, it would lead to abnormally high-level cytokines that in turn caused joint inflammation and bone destruction. These studies suggest that cfDNA may be a target for RA therapy. Recently, Sullenger et al. demonstrated that cationic polymers can be used to neutralize cfDNA to inhibit inflammation in an acute liver injury model and in alleviating symptoms of systemic lupus erythematosus (SLE).

Hypothesizing that blocking the TLR activation may reduce inflammation in RA, we propose the use of cationic polymers to scavenge damage-associated molecular patterns (DAMP) molecules as a new strategy to treat RA. In addition, we opt to focus on cationic nanoparticles (cNP) instead of soluble polycations because of a potentially higher nucleic acid (NA) scavenging capacity and a more favorable biodistribution in the inflamed joints (Fig. 1a). In the development of RA, inflammation induces angiogenesis to form a leaky vasculature in the inflamed joints. We show that cNP composed of the diblock copolymer of poly(lactic-co-glycolic acid) (PLGA) and poly(2-(diethylamino)ethyl methacrylate) (PDMA) with a size around 40 nm have a high DNA-binding affinity, which in turn efficiently inhibit primary SFMC and FLS activation by cfDNA from RA patients (Fig. 1a). Intravenous injection of cNP into a Cpg-induced mouse or collagen-induced arthritis (CIA) rat model would relieve RA symptoms with respect to ankle and tissue swelling and reduce bone and cartilage damage. The cNP are effective in treating the CIA model at both the early stage, as well as the established stage of RA progression. Treatment with cNP also leads to partial mobility recovery in the CIA-induced RA rats. The favorable therapeutic outcome is corroborated with determination of intracellular trafficking, biodistribution, cfDNA levels in systemic circulation and inflamed joints, and cytokine levels in the joints.
protein expression level in the downstream of the signal pathways by Western blotting. CpG 2006 activates immune cells through the MyD88-NFκB signaling pathway. Once TLR9 senses NAs, the adaptor protein MyD88 is activated to form a homo-oligomeric signaling complex, which in turn, recruit mononuclear cells to the synovium to damage the cartilage and bones. After intravenous injection of cNP to scavenge cDNA, cytokines are down-regulated and symptoms are alleviated. b DNA-binding efficiency of PDMA and cNP in PBS and 10% FBS at 37 °C. c Inhibition of TLR activation in Ramos Blue™ cells using the QUANTI-Blue™ assay. Statistical significance was calculated by one-way ANOVA with the LSD post-test. ***P < 0.001 versus 0 μg/mL materials + CpG. ###P < 0.001 between two groups. In b and c, data are presented as the mean ± s.e.m.

**Fig. 1** cNP shows high DNA-binding ability and efficient inhibition of TLR9 activation. a Mechanism of using cNP to scavenge cDNA to inhibit inflammatory response for treating RA. cNP with PDMA corona is formed from PLGA-b-PDMA block copolymer self-assembly. In the RA animal models, damage-associated molecular patterns (DAMP) including cDNA stimulate immune cells to release cytokines, which, in turn, recruit mononuclear cells to the synovium to damage the cartilage and bones. After intravenous injection of cNP to scavenge cDNA, cytokines are down-regulated and symptoms are alleviated. b DNA-binding efficiency of PDMA and cNP in PBS and 10% FBS at 37 °C. c Inhibition of TLR activation in Ramos Blue™ cells using the QUANTI-Blue™ assay. Statistical significance was calculated by one-way ANOVA with the LSD post-test. ***P < 0.001 versus 0 μg/mL materials + CpG. ###P < 0.001 between two groups. In b and c, data are presented as the mean ± s.e.m.

**Fig. 2** cNP inhibits intracellular agonists to TLR9. In the above experiments, cationic materials were added at the same time with the CpG to the cell. In the non-idealized situation, the pathogenic NA would already be at the tissue and interact with immune cells when the scavengers reach them. To mimic this scenario, we first incubated the reporter cells with CpG and then the cells were washed thoroughly to remove any free extracellular CpG before the addition of the materials. Fig. 2a showed that, at the concentration of 25 μg/mL, cNP could decrease the activation of TLR9 to ~80%, whereas PDMA showed no effect. However, at dose of 50 μg/mL, both materials decreased the activation of TLR9 to 40%, implying that the TLR9-bound CpG could be captured competitively due to the strong binding affinities from the materials. This result was further confirmed by a different cell, the mouse macrophage cell line RAW264.7, at dose of 25 μg/mL, using the same protocol (Fig. 2b). Still, cNP showed better inhibition activity than that of PDMA. These results suggest that the cationic materials could enter the cells and capture the cDNA from TLR9 in the endolysosomal compartment. To prove this, we conducted an intracellular trafficking study with Cy5-labeled CpG 1826, selective to mouse TLR9, and the materials in RAW264.7 cells. Localization of CpG in the endolysosome suggests TLR9 recognition (Fig. 2c and Supplementary Fig. 6a). When FITC-labeled cationic materials were added and their intracellular location monitored over a 12-h period (Supplementary Fig. 6a for different time), the PDMA intensity increased but dispersed in...
the nucleus, endolysosome, and cytoplasm (Fig. 2c). In contrast, the cNP showed preferential localization in the endolysosome, where the CpG remained unchanged. Quantitative colocalization ratio of cNP in endolysosome at different time is much higher than that of PDMA (Supplementary Fig. 6b). This specific localization of cNP and CpG in the endolysomal compartment may help explain the therapeutic effect in the in vivo study.

We next investigated whether the cationic materials could also enter the primary SFMC and FLS from RA patients, to inhibit the inflammatory response by endogenous cfDNA also from the same patients. By transducing into the cells with Lipofectamine® 2000 (Lipo), cfDNA induced significantly more TNF-α and IL-6 from SFMC and FLS, respectively (Fig. 2d, e), while the materials alone showed no significant response (Supplementary Fig. 2d, e). In the presence of either PDMA or cNP, the production of cytokines was inhibited obviously. The decrease of cytokines by cNP treatment was even more obvious than that of PDMA.

**cNP alleviates symptoms of CpG-induced acute arthritis.** One day after injection of 6 μg CpG into the articular cavity of mice, acute arthritis was set as manifested by swollen hindpaws. The animals were then treated with intravenous injection of cNP at a daily dose of 12.5 mg/kg for the following 7 days (Fig. 3a). Hindpaw swelling of the mice decreased gradually (Fig. 3b and Supplementary Fig. 7a). The diameter of the inamed ankles

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**Fig. 2** cNP better inhibits intracellular CpG to activate TLR9 via a stronger interaction with CpG. **a** Inhibition of TLR9 of Ramos Blue™ cells due to intracellular DNA scavenging by cationic materials. First, 1 μM CpG 2006 was added to Ramos Blue™ cells. After incubation for 4 h, cationic materials were added. Statistical significance was calculated by one-way ANOVA with the LSD post-test. **b** Cationic materials inhibited TNF-α expression in RAW264.7 cells. Firstly, RAW 264.7 cells were incubated at 37 °C for 4 h with 1 μM CpG 1826, then the medium was replaced with the medium containing cationic materials. After 24 h, CpG up-regulated TNF-α expression. After incubating with PDMA or cNP, TNF-α expression decreased. **c** Enlarged images show intracellular localization of intracellular CpG and cationic materials in RAW264.7 cells after 8 h incubation (Bar: 5 μm). Colocalization of CpG and cationic materials showed up as white spots, which are marked by the arrow. D indicates DAPI, L indicates LysoTracker, P indicates polymer, and C indicates CpG. **d** and **e** Cationic materials inhibited intracellular cfDNA to stimulate SFMC (**d**) and FLS (**e**). cfDNA and SFMC were collected from the synovial fluid of the same patient. In **b** and **d**, 25 μg/mL of the materials; in **e** 0.5 μg/mL of the materials. In **b**, **d**, and **e** statistical significance was calculated by one-way ANOVA with the LSD post-test, *P < 0.01, **P < 0.001 versus 0 μg/mL materials + CpG, ###P < 0.001 between two groups. Data are presented as the mean ± s.e.m.
cNP relieves symptoms of CIA rats in therapeutic treatment. The above results show that cNP inhibited the inflammatory response of CpG-induced acute arthritis model by specifically blocking the TLR9 activation. However, in the CpG-induced arthritis model, T and B cells are not required in model developing, which is different from that of human RA. To investigate whether the cationic materials can perform in a more mimetic model, we conducted the studies in the CIA model, whose pathological changes.

To assess synovitis, another common symptom of RA in addition to bone erosion, we applied MRI to evaluate the soft tissue disorder around the joints. The MRI images show the appearance of effusion in the knee and ankle joints, and suprapatellar bursa, as well as swelling of one popliteal lymph node in the model (Fig. 3e and Supplementary Fig. 7c). Whereas, after treatment, the effusion decreased and the popliteal lymph node shrank to a size that was undetectable. The pathological assessment also indicates that the inflammatory cell infiltration at the synovium and articular cavity in the model was decreased after cNP treatment, with low histology scores close to the normal group (Fig. 3f and Supplementary Fig. 7d, e).

The CIA rat model is associated with symptoms of swelling and erythema in ankle and paw joints, as well as bone erosion due to inflammation in synovial cavity. For the early stage treatment, 13 days after the disease induction and when symptoms appeared, the animals were treated with a daily intravenous injection of the cationic materials for 15 days (Fig. 4a). A clinical scoring was used to evaluate the treatment effect. The hindpaws of the model groups swelled gradually and reached a maximum of 2.2 mL at day 21, while the forepaws swelled more gradually (Fig. 4c). At a dose of 12.5 mg/kg, both the cNP and PDMA groups showed reduced swelling in hindpaws and forepaws (Fig. 4c and Supplementary Fig. 8a). Beyond day 20, the cNP groups showed a clearer inhibition of the hindpaw swelling than the PDMA group. At a dose of 25 mg/kg, both the cNP and PDMA groups showed reduced swelling in hindpaws and forepaws (Fig. 4c). At a dose of 12.5 mg/kg, both groups showed improved clinical scores in both of their hindpaws and forepaws. Thus, for the cNP groups, the 25 mg/kg dose was more effective than the 12.5 mg/kg dose.

The micro-CT analysis of inflamed ankle joints at day 29 showed a serious bone erosion in the model group, with the BMD dropping from 1564 to 1246 mg/cm³ (Fig. 5a, b). Treatment with 12.5 mg/kg of PDMA between days 13 and 28 saw a small improvement with the BMD to 1309 mg/cm³. On the contrary, the macrophages play critical roles in tissue damage during disease development. We evaluated the performance of cNP therapeutic treatment in both early and established stages of the model, aiming to assess the translational potential of cNP as a new strategy for treating RA (Fig. 4a). Firstly, the injection dose was optimized (Fig. 4b). At a dose of 25 mg/kg, all rats injected with PDMA died immediately, whereas cNP resulted in a death rate of 25%. At half the dose of 12.5 mg/kg, all rats in the cNP group survived while the PDMA group resulted in a death rate of 35.7%. Thus, the dose of 12.5 mg/kg for both cNP and PDMA and also 25 mg/kg for cNP were studied in four groups.

Fig. 3 cNP improves the arthritic condition of BALB/c mice induced by CpG. a Experimental schedule of the mice study. Acute arthritis developed one day after articular injection with CpG. Then arthritis mice were injected i.v. daily with 12.5 mg/kg cNP from day 1 to day 7. Further study was performed after sacrificing mice at day 8. b Right ankle joint swelling of all mice was evaluated daily by measuring the diameter of the right ankle with a digital caliper (n = 5). c Analysis of BMD of mice ankle joint from Micro-CT data. Statistical significance was calculated by one-way ANOVA with the LSD post-test, ***P < 0.001 versus model group, ###P < 0.001 versus normal group. d Representative micro-CT images of the ankle joint of BALB/c arthritis mice after administration for 7 days with a resolution of 19 μm. 3D images were reconstructed using Inveon Research Workplace. Various levels of bone destruction in calcaneus are shown in red circles. e Representative T2-weighted MRI images of ankle joint of mice at day 7. The swollen popliteal lymph node (red circle), effusion in the joint (single arrow), and suprapatellar bursa (double arrow) appeared in the model, while treatment with cNP efficiently prevented these pathological changes. f Representative HE staining of ankle joints of mice after administration for 7 days (×200). Some inflammatory cell infiltration (single arrow) appeared in the articular cavity of model. In b and c data were presented as the mean ± s.e.m.
the BMD of the cNP groups at low and high doses were 1391 and 1441 mg/cm³, respectively, closer to the normal group (Fig. 5b) and demonstrating clear inhibition of bone erosion. The trabecular parameters also confirmed that cNP group showed better anti-inflammatory effect than the PDMA group and that the higher dose was more effective (Supplementary Fig. 8b). In addition, MRI images taken at day 24 showed a large amount of joint effusion and severe soft tissue swelling in the ankle and foot of model rats (light area in Supplementary Fig. 8c). The swelling was decreased by treatment with 25 mg/kg of cNP. Histological analyses of various joints (Fig. 5c for knee joints and Supplementary Fig. 8d for other joints) indicated a severe bone and cartilage damage accompanied by a large amount of infiltration of mononuclear cells in the model rats. In the low-dose treatment groups, although there was still evidence of inflammatory cells in the synovium in all groups, the cNP-treated rats showed reduced bone and cartilage damage compared with the rats in the model group and the PDMA group. In the high-dose cNP group, the protection from cartilage and bone damage was more obvious compared with other groups when treatment was initiated at the early stage, and could be seen even with treatment initiated at the established stage compared with the model group.

We next evaluated the therapeutic effect of cNP for the model rats at an established stage. When the paw swelling peaked at day 21, a daily dose of cNP at 25 mg/kg was injected intravenously for 7 days (Fig. 4a). Surprisingly, the swollen hindpaws and forepaws both shrunk considerably and even recovered to the same extent as the early treatment (Fig. 4c, highlighted by thick green curve), implying that the inflammation was effectively controlled. Furthermore, the erosive bone damage was arrested, as evident by the BMD value of 1442 mg/cm³ (Fig. 5b) and the trabecular parameters (Supplementary Fig. 8b). The histological study also showed that, compared with the model group, the synovium and other soft tissues were restored even in the treatment at the established stage, and histology scores of knee joints, ankle joints, digital joints and wrist joints were obviously lower after treated by cNP (Fig. 5c and Supplementary Fig. 8d, e).

Advanced RA patients often lose their agility and it is very challenging to restore even the basic mobility. We finally evaluated the mobility of the model and treated CIA rats in a rotational cage test (Fig. 5d, e and Supplementary Movie 1). We chose the most relevant cNP group, 25 mg/kg dose in the treatment of the rats at the established stage, for this behavioral assay. In contrast to the untreated CIA rats that could barely move, the cNP group could manage an average rotational speed of 3.5 rpm, while the normal group could stand the rotational speed up to 7 rpm.

To understand the different effects of the cationic materials and the mechanism of the therapeutic effects, we measured the biodistribution of the cationic materials, cfDNA levels in circulation and the synovial joints, and cytokine profiles in the joints. We studied the biodistribution of the cationic materials in the joints with time using in vivo near-infrared fluorescence (NIRF) imaging (Supplementary Fig. 9a), and determined their biodistribution in the main organs and joints using ex vivo NIRF imaging (Fig. 6a). In normal rats, the biodistribution patterns of the PDMA and cNP groups differed only in the lung, liver, kidney, and spleen but not in the joints (Supplementary Fig. 9b). In the model group, the PDMA accumulated mainly in the lung,

**Fig. 4** cNP better alleviates the swelling of joints in the CIA model in both early and established therapeutic treatment. a Experimental schedule of the CIA model study. After immunization with collagen type II and Freund’s adjuvant twice (at day 0 and day 7), the CIA model was developed. Then CIA rats were i.v. injected with cationic materials from day 13 to day 28 for the early-stage treatment, or i.v. injected with cNP from day 21 to day 28 for the established-stage treatment. Photos and blood were taken regularly during therapy. Further study was performed after the rats were sacrificed at day 29. b Survival curve of different treatment groups from day 13 to day 28 after disease progression. Initial number of rats: n = 5 for PDMA, 25 mg/kg; n = 16 for cNP, 25 mg/kg; and n = 13 for cNP, 12.5 mg/kg. Statistical significance was calculated by a log-rank (Mantel-Cox) test, *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001. c Hindpaw swelling (ci), clinical score of hindpaw (cii), and forepaw (ciii) of rats treated in the early stage or the established stage of RA progression (thick green curve). (ci) Average hindpaw swelling was determined daily using a plethysmometer, and (cii and ciii) the average clinical score of the hindpaws and forepaws of all rats was evaluated. n = 10 for model, PDMA (12.5 mg/kg), cNP (12.5 mg/kg), and cNP (25 mg/kg) groups. n = 5 for the normal group and cNP groups (25 mg/kg d21–28). Thick green curve is highlighted for the established-stage treatment with cNP (25 mg/kg). Data were presented as the mean ± s.e.m.
liver, and kidneys at 4 h, and relatively less in knees and paws. In contrast, cNP had a much higher accumulation in the knees and paws but much less in the other organs (Supplementary Fig. 9c). Moreover, cNP showed a longer retention in the paws but much less in the other organs (Supplementary Fig. 9c). In contrast, cNP had a much higher accumulation in the knees and liver, and kidneys at 4 h, and relatively less in knees and paws. In contrast, cNP had a much higher accumulation in the knees and paws but much less in the other organs (Supplementary Fig. 9c).

We also measured the mRNA level of key inflammatory cytokines, including TNF-α, IFN-α, IL-6, and matrix metalloproteinase (MMP) 3 in the shin bones of CIA rats after administration for 15 days (Fig. 6d). Consistent with the lower cfDNA levels in the synovial joints, the cytokine and matrix metalloproteinase (MMP-3) levels related to the pattern recognition receptor (PRR) signaling pathways in the cNP group were all significantly reduced relative to the model group, and close to the normal group. In contrast, the PDMA group could show reduction only in IFN-α. The results have been confirmed by immunohistochemical analysis, the number of TNF-α, IL-6, MMP-3 immunoreactive cells were low in both cationic materials groups, among them cNP showed more efficiently inflammatory inhibition (Fig. 6e and Supplementary Figure 10).

**Discussion**

Cationic polymers have been extensively applied to deliver NA for nonviral gene therapy for over two decades. Since 2009 Sullenger et al. have instead proposed to use cationic polymers to compete off aptamers or remove proinflammatory DAMP for therapeutic purposes. They have recognized as DAMP or pattern-associated molecular patterns (PAMP) by PRR, such as the TLR in immune cells. Scavenging these DAMP molecules may limit inflammation. Using these nucleic acid-binding polymers (NABP) to remove the DAMP molecules, we had demonstrated that the scavenging approach...
Fig. 6 cNP accumulates more in inflamed joints, and more efficiently reduce cfDNA and proinflammatory cytokine expression in vivo than soluble polycations. a Ex vivo near-infrared fluorescence (NIRF) imaging of normal rats and CIA rats (clinical scoring 2) after i.v. injection of PDMA and cNP. Legend: A. thymus; B. heart; C. lung; D. liver; E/F. kidneys; G. pancreas; H. spleen; I. bladder; J/K. forelimbs; L/M. hindlimbs. b Changes of cfDNA concentration in the serum of rats treated with PDMA and cNP. c Concentration of cfDNA in knee joints after treatment with PDMA and cNP at day 29 after first immunization. d mRNA level of cytokines, TNF-α, IFN-α, and IL-6, as well as MMP-3 of tissue at day 29 after the first immunization. Relative mRNA expression of the cytokines and MMP-3 was normalized to GAPDH. In b, c, and d, data were presented as mean ± s.e.m. Statistical significance was calculated by one-way ANOVA with the LSD post-test, *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001. e Detection of TNF-α, IL-6, and MMP-3 immunoreactive cells in synovial tissue of different groups. Synovial tissues of knee joints of different groups at day 29 were stained with anti-TNF-α, anti-IL-6, and anti-MMP-3 antibodies, respectively (*×400). The inset showed the enlarged detail (*×1000).
works in animal models of CpG-induced or RNA-induced acute liver injury\textsuperscript{19}, thrombosis\textsuperscript{19}, and SLE\textsuperscript{20,30}. To reduce toxicity, we had immobilized the NABP on fibrous membrane to remove the DAMP molecules in a local\textsuperscript{31} or ex vivo manner\textsuperscript{32}. Motivated by the high NA-binding capacity of the immobilized configuration of NABP in these recent studies, we propose to use cNP as a DAMP scavenger, which has not been studied before, for RA treatment. Previously, Chauhan et al. observed that poly(amidoamine) dendrimers showed an anti-inflammatory activity toward arthritis, but no mechanism was discussed\textsuperscript{33}. Dong et al. found that cationic dextran and PEI enhanced systemic concentrations of IL-12 and IFN-γ, and proposed that they in turn prevented neutrophil infiltration to the inflammatory sites in an adjuvant-induced arthritis (AIA) mouse model\textsuperscript{34}. In these two studies, only soluble cations were used. Finally, Hayden et al. reported that azabiphosphonate (ABP)-capped dendrimers could ameliorate inflammation in a IL-1ra\textsuperscript{−/−} murine model and a K/BxN serum transfer model to reduce damage in bone and cartilage\textsuperscript{35}. The proposed mechanism was to inhibit inflammation activation via interactions with monocytes. Anionic in nature, this polymer would likely act through a different mechanism than what we proposed in this study.

Here, we established that the nanoparticulate format of NA-scavenger could achieve what the soluble counterpart could not in treating RA in rat models. We designed the PLGA-$b$-PDMA because PDMA by itself is a potent polycation used in nonviral gene delivery, and PLGA provides the hydrophobic core to form cNP. The binding of NA to cNP stems from charge interaction and we could optimize the NA-scavenging performance of the cNP by varying the molecular weight of PDMA. Moreover, the cNP would also likely interact with NETs and these interactions warrant further studies. The cNP should also bind to negatively charged proteins although its interaction with NA should be stronger than that with proteins. Therefore, in the presence of serum, cNP may still bind cfDNA efficiently as shown in this work (Fig. 1b). We have shown that cNP may have unique scavenging properties due to its different extracellular and intracellular distribution characteristics from soluble polycation PDMA; it has higher cfDNA scavenging efficiency in the circulation and inflamed joint, and it shows a more effective reduction of DNA-mediated immune stimulatory activities in vitro and in vivo. This indicates that cationic nanostructured materials can modulate inflammation in an unexpected manner, which has not been reported before.

Although cNP might inhibit all NA-mediated activation of TLR, we have only focused on cfDNA in this study, because its high concentration in RA patients in both the serum and the synovial fluid has been documented\textsuperscript{8,9}. In contrast, the concentration of dsRNA in the serum and the synovial fluid of RA patients is much lower than the cfDNA concentration\textsuperscript{36}, prompting us to overlook this parameter that may warrant future scrutiny. Using a known CpG that binds TLR9, we demonstrated that the cNP was more effective in blocking the TLR9 activation by the colocalization study (Fig. 1c and Fig. 2a). We then confirmed that cfDNA isolated from the synovial fluid of RA patients could stimulate human primary SFMC and FLS to up-regulate the synthesis of proinflammatory cytokines TNF-α and IL-6, respectively (Fig. 2d, e). Interference with cNP would inhibit the stimulation, with an efficiency higher than that of the soluble polycationic counterpart. This may be attributed to the cooperative effect of the cationic corona in binding the anionic cfDNA (Fig. 1b). This is consistent with our understanding of the intracellular trafficking of polyplexes and lipoplexes in nonviral gene delivery, that after internalization they would be sequestered into the endolysosomal compartments, where TLR9 resides in the vesicle membrane.

Using the CpG-induced acute RA model in mice, we confirmed that the scavenging approach using cNP would also work in vivo as supported by clinical scoring, micro-CT, MRI, and histology (Fig. 3). We then elaborated the merits of the concept with a clinically more relevant CIA model in rats. In addition to investigating the earlier stage therapy where the treatment was initiated at the onset of RA symptoms, we also evaluated the efficacy in the model at an established stage where treatment started one week after the onset and when the swelling in the hind paws peaked. In both cases, the treatment produced a positive effect (Fig. 4 and Fig. 5). This culminated in a rotational cage assay where the cNP-treated animals could recover partial mobility.

In all rat experiments, the cNP consistently out-performed the soluble counterpart. This may be attributed to a more favorable biodistribution of the cNP (Fig. 6a and Supplementary Fig. 9) due to the leaky microvasculature in the inflammatory sites\textsuperscript{21,22}. In normal rats, free PDMA and cNP showed little difference in their accumulation in the joints and paws; the difference is in liver and the spleen as one would expect. In RA rats, both cationic materials have a higher accumulation in the joints and paws relative to normal rats, suggesting that inflammation does affect their biodistribution. Comparing the biodistribution of both materials in normal versus RA rats, there was also higher accumulation in the liver and kidney of the RA rats, suggesting those are also tissues with either inflammation or higher cfDNA level. Comparing the biodistribution of cNP versus PDMA in RA rats, more cNP had gone to the joints and paws, with a corresponding decrease in the lung, liver, and kidney. But there was an increase of cNP accumulation in the liver at 24 h versus 4 h as the cNP were cleared from the paws. Therefore, the biodistribution data elucidate why the cNP is a better performer in the RA model. However, recent findings indicate that the leaky vasculature observed in the tumor tissue of rodent is often irreproducible in human\textsuperscript{37}. One must therefore be cautious in interpreting the importance of leaky vasculature in treating RA until this phenomenon is also confirmed in human.

The biodistribution on mechanistic speculation of how cNP is a more effective inflammation inhibitor than PDMA in vivo is nevertheless corroborated with the biochemical data. The cNP were more effective in scavenging the cfDNA in both the systemic circulation and the inflamed joints (Fig. 6b, c), consistent with the in vitro-binding data. The almost complete reduction of systemic cfDNA to the normal level by cNP is probably aided by the longer retention of the cNP in various tissues directly exposed to the systemic circulation. It is understandable that the reduction of cfDNA in the inflamed joints would not be so pronounced because of transport barriers to the cNP. Compared with PDMA, the cNP was also more effective in reducing the TNF-α expression in the inflamed joints (Fig. 6d). Taken together, the mechanistic studies validated the superior performance of the cNP in treating RA.

Toxicity of cationic materials is a concern in systemic applications. At a dose of 25 mg/kg, cNP produced the greatest relief of RA symptoms and did not induce thrombosis, whereas PDMA showed serious acute toxicity and caused blood coagulation and edema fluid in the lung (Supplementary Fig. 11a, b). Therefore, cNP accumulated less in lung and did not cause pulmonary edema. To further analyze the long-term toxicity of the cationic materials, we performed histological analysis of the heart, liver, spleen, lung and kidney of CIA rats after i.v. injection of cationic materials for 15 days on a daily basis. While PDMA caused different degrees of lesions in liver, spleen, lung, and kidney, the cNP-treated group showed no damage on various major organs by gross observation (Supplementary Fig. 12a). Also, the hepatotoxicity of cNP as reflected in ALP, ALT, AST levels and its
nephrotoxicity reflected in creatinine, urea, and uric acid before and after treatment were close to the normal group at the lower dose of 12.5 mg/kg (Supplementary Fig. 12b). The relatively low toxicity of cNP may be attributed to a biodistribution profile different from that of the soluble PDMA and an efficient NA scavenging capacity due to a multi-valent cationic corona. The latter allows the cNP to be administered at a lower dose in terms of charge density per mass compared with the soluble PDMA.

Motivated by the findings that cDNA from RA patients would activate primary SMC and FLS, we show that cNP can be used to scavenge pro-inflammatory NAs to treat experimental RA. While the balance between toxicity and efficacy remains to be optimized to determine the translational potential of this therapeutic strategy for RA, this work suggests a new direction for nanomedicine. It is the opposite of using cationic materials to deliver NAs. All the innovations in nonviral gene delivery can be exploited for cNP designs to achieve efficacious and safe removal of pathogenic DAMP molecules. This new strategy may be widely applicable to tackle inflammatory diseases initiated by inappropriate activation of the TLR pathways.

Methods

Chemical reagents. Dodecanol terminated PLGA (composition 50:50, Mw = 8000, Mn = 12 000 calculated by ’H NMR spectrum, Daigangbo Company, China) was dried byzeotropic distillation in the presence of toluene. 2-(Dimethylamino)ethyl methacrylate (DMA, purity > 98.5%, TCI), N,N,N’,N’-penta- methylidienetriamine (PMDETA, purity > 98%, Acros), cuprous bromide (CuBr, purity > 99%, Aladdin, China), triethylamine (Guangzhou Chemical Reagent, China), bis (tert-butyl)carbonate (Adamas), trifluoroacetic acid (TFA, purity > 99%, Aladdin, China), and ethyl a-bromoisobutyrate (EBiB, purity > 98%, TCI) were commercial products without further treatment. Fluorescein isothiocyanate (FITC, Aladdin, China) was used in its stock solution. 10 mg/mL, in DMSO. Alexa Fluor 750 NHS Ester (succinimidyl ester) (AF750-NHS, Invitrogen) was dissolved in DMSO to prepare a stock solution of 10 mg/mL. Boc-protected AEMA monomer (AEMA-Boc) was used for copolymerization with DMA in order to introduce a fluorophore to the polymer chain. It was synthesized according to literature38. The purity was proved by1H NMR (Bruker AVANCE III 400 MHz, Figure 1c). The first step was to synthesize PLGA-Br macroinitiator. 3 g (0.25 mmol) of PLGA was dissolved in 18 mL of CH2Cl2, and then 60 μL (0.4 mmol) of CuBr was charged. The solution was being cooled to -20 °C by dry ice. 13.3 mg (0.073 mmol) PMDETA, 6.35 g (40.4 mmol) DMA, 84 mg (0.36 mmol) AEMA-Boc, and 2.5 mL of anisole were charged into a dried polymerization tube and the mixture was deoxygenated by three freeze-pump-thaw degassing cycles. The polymerization was conducted in an oil bath at 70 °C for 17 h and was terminated by cooling and exposing to the air. The resulting mixture was diluted with 40 mL of THF and the solution was then passed through a short alkaline alumina column to remove the copper residue. After evaporated to dryness, the crude product was dissolved in 20 mL of THF and precipitated dropwise into 200 mL of cool hexane and repeated three times. The collected product was dried in vacuum for 24 h at room temperature. The monomer conversion rate was 85% as determined by composition of the monomer and PDMA in the polymerization mixture from1H NMR (Bruker AVANCE III 400 MHz, in CDCl3) spectrum δ/ppm: 6.1 and 5.6 (2. H, CH2 = C), 4.3 (1 H, NH = C = O), 4.2 (2 H, NCH2CH2O), 3.4 (3 br. 2 H, NCH2CH2O), 1.9 (1 S, CH3-C), 1.5 (1 S, 9H, (CH3)3-C).

Characterization of cNP. The size and polydispersity index (PDI) of cNP in 10 mM PBS buffer (pH = 7.4) were measured in triplicated by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS) at 25 °C. The number average diameter of cNP was 44.0 nm with PDI being 0.149 (Supplementary Figure 1f). The zeta potential of cNP in PBS (pH = 7.4) was 18.5 ± 3.7 mV measured by Malvern Zetasizer Nano ZS. Furthermore, the morphology of cNP was characterized by transmission electron microsphere (TEM, JEM-14000+) (Supplementary Figure 1g).

Synthesis of PDMA470. PDMA470 was prepared by atom transfer radical polymerization (ATRP) (see schematic syntheses in Supplementary Figure 1b). 14.3 mg (0.046 mmol) of PLGA was dissolved in 18 mL of CH2Cl2, and then 60 μL (0.4 mmol) of CuBr was charged. Then 60 mg (0.36 mmol) of bromoisobutyryl bromide in 2 mL of CH2Cl2 was added dropwise. The solution was stirred in an ice bath for 4 h and then was brought to 30 °C for 20 h. The reaction mixture was washed with aqueous solutions of 1 M HCl, saturated NaHCO3, and saturated NaCl. The organic phase was washed with magnesium sulfate, concentrated to 10 mL, and then precipitated twice into 100 mL mixture of cold diethyl ether and methanol (volume ratio = 2:1). The product was isolated by filtration and dried in vacuum for 24 h at room temperature. The second step was the ATRP block copolymerization. 0.55 g (0.046 mmol) PDMA470, 0.823 g (0.046 mmol) of PLGA, and 0.34 mg (0.046 mmol) of CuBr in dry THF (0.4 mL) was stirred for overnight at room temperature. The solution was added with deionized water and concentrated to 2.5 mg/mL using ultrafiltration tube (Amicon® Ultra-4 10K, Millipore). Preparation of cNP by self-assembly. PLGA-B-PDMA470 diblock copolymer was dissolved in THF at a concentration of 12.5 mg/mL. Under ultrasound (Ultrasound Processor, Sonics VX105), the sonication of block copolymer was dropped slowly to an aqueous solution of hydrochloric acid at pH = 3 until the polymer concentration reached 2.5 mg/mL. The solution was dialyzed against a diluted hydrochloric acid at pH=3 for 12 h to remove the THF. Then, the pH was adjusted to 7.4 by adding a mixture of Na2HPO4·12H2O and KH2PO4, and the phosphate concentration of the final PBS buffer was 10 mM.

Bio-reagents. Phosphate buffered solution (PBS, pH = 7.4), Dulbecco’s modified Eagle medium (DMEM), Iscove’s modified Dulbecco’s medium (IMDM), Roswell Park Memorial Institute 1640 Medium (RPMI 1640), and fetal bovine serum (FBS) were purchased from United States Origin, Gibco. Calf thymus DNA was purchased from Sigma Aldrich and its stock solution, 1 mg/mL, was prepared in PBS. CpG 2006, CpG 1668, CpG 1826, and Cy5-CpG 1826 were purchased from United States Origin, Gibco. Other reagents were purchased from Sangon Biotech, China. Preparations of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Sangon Biotech, China. Primers were synthesized by Sangon Biotech, China.

Figure 1a. Synthesis of PDMA-PMDETA diblock copolymers. PLGA-B-PDMA was prepared by ATRP in two steps (see schematic syntheses in Supplementary Figure 1c). The first step was to synthesize PLGA-Br macroinitiator. 3 g (0.25 mmol) of PLGA was dissolved in 18 mL of CH2Cl2, and then 60 μL (0.4 mmol) of CuBr was charged. The solution was then cooled to -20 °C by dry ice. 13.3 mg (0.073 mmol) PMDETA, 6.35 g (40.4 mmol) DMA, 84 mg (0.36 mmol) AEMA-Boc, and 2.5 mL of anisole were charged into a dried polymerization tube and the mixture was deoxygenated by three freeze-pump-thaw degassing cycles. The polymerization was conducted in an oil bath at 70 °C for 17 h and was terminated by cooling and exposing to the air. The resulting mixture was diluted with 40 mL of THF and the solution was then passed through a short alkaline alumina column to remove the copper residue. After evaporated to dryness, the crude product was dissolved in 20 mL of THF and precipitated dropwise into 200 mL of cool hexane and repeated three times. The collected product was dried in vacuum for 24 h at room temperature. The monomer conversion rate was 85% as determined by composition of the monomer and PDMA in the polymerization mixture from1H NMR (Bruker AVANCE III 400 MHz) spectrum in CDCl3. Thus, the degree of polymerization was obtained as 470 and the molecular weight (Mn) of PDMA was calculated to be 71000 Dalton. The1H NMR spectrum of the pure product was shown in Supplementary Figure 1f. The1H NMR spectrum of the pure product was shown in Supplementary Figure 1f. The1H NMR spectrum of the pure product was shown in Supplementary Figure 1f. The1H NMR spectrum of the pure product was shown in Supplementary Figure 1f. The1H NMR spectrum of the pure product was shown in Supplementary Figure 1f.


**IL-27**

IL-27 is also known to be involved in the regulation of immune responses. IL-27 can be produced by a variety of cell types, including dendritic cells (DCs), T cells, and macrophages. It plays a role in the induction of Th1 and Th17 immune responses and has been found to be expressed in conditions such as autoimmune diseases, infection, and cancer.

**Release of Cytokines**

Cytokines are a group of signaling molecules that play a key role in the regulation of immune responses. They are released in response to various stimuli, such as infections, and can activate or suppress immune cells. Cytokines such as IL-12, IL-18, and TNF-α are important in the induction of Th1 responses, while IL-4 and IL-13 are crucial for Th2 responses.

**Activation of DCs**

DCs are professional antigen-presenting cells that play a critical role in the induction of adaptive immune responses. They are responsible for presenting antigens to T cells and initiating the immune response. DCs are activated by various stimuli, including cytokines and pathogen-associated molecular patterns (PAMPs). Activated DCs can migrate to the lymph nodes and present antigens to T cells, leading to the induction of an immune response.

**Role of IL-27 in Autoimmune Diseases**

IL-27 has been shown to play a role in the regulation of autoimmune responses. It can promote the expansion of regulatory T cells and suppress the activity of Th1 and Th17 cells. This makes IL-27 a potential target for the treatment of autoimmune diseases.

**Conclusion**

In conclusion, IL-27 is a cytokine that is involved in the regulation of immune responses. It is produced by a variety of cells and plays a role in the induction of Th1 and Th17 responses. Its role in autoimmune diseases is still being explored, but it holds promise as a potential target for the treatment of these diseases.

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**IL-27**

IL-27 is a cytokine that plays a critical role in the regulation of immune responses. It is produced by a variety of cell types, including dendritic cells (DCs), T cells, and macrophages. IL-27 is involved in the induction of Th1 and Th17 responses, as well as the suppression of Th2 responses. It is also important in the regulation of autoimmune diseases, as it can promote the expansion of regulatory T cells and suppress the activity of Th1 and Th17 cells. IL-27 is a potential target for the treatment of autoimmune diseases.
Cytochrome concentration analysis with ELISA. The concentration of TNF-α in the culture supernatants of RAW cells was determined with Mouse TNF-α ELISA Kits. The results, the concentrations of TNF-α and IL-6 in the culture supernatants of primary cells from patients were determined with ELISA Kits using human TNF-α and IL-6 ELISA Kits.

Cellular colocalization of CpG and cationic materials. In the NUNC™ LabTek™ eight-well plates, 2 x 10⁶ cells/well of RAW264.7 cells were cultured overnight. Then two experiments were conducted. The first one was to test if the cationic materials could reduce the cell uptake of extracellular NA. 1 μM of Cy5-CpG 1826 with 1.0 μg/ml cationic materials were added into the new culture media to replace the old media and incubated for 12 h. The second one was to test if the cationic materials could bind intracellular NA agonist and inhibit its stimulation to IL-19. After washing three times with fresh culture medium, 1 μM of Cy5-CpG 1826 was added into the culture media and incubated for 4 h. The excessive CpG was removed by washing three times with PBS, then the cationic materials labeled with FITC in medium (1.0 μg/ml) were added. After 4, 8, 12 h, the cells treated in these two experiments were stained with Lysotracker Red DNA-99 and DAPI for confocal microscopic observation (Leica S8P). Mean fluorescence intensity of Cy5-CpG 1826 was the optical density of Cy5-CpG 1826 per cell, as calculated by Image J software. Colocalization ratio of Cy5-CpG 1826 and FITC-cationic materials was the ratio of colocalization area (the area of white spots) and foreground area (the area of green and purple spots), calculated by Leica Application Suite.

Animal model induction and treatment. The acute arthritis model was induced with CpG 1666R45. Six micrograms of CpG was injected intra-articularly into the right knee joint of BALB/c mice. Onset of arthritis occurred one day later, and the arthritic mice were evenly divided into model and treatment groups randomly. There were three groups including normal control, model control and cNP treated group, with 6 mice per group. For cNP treated group, a solution of cNP in PBS (2.5 mg/ml, 100 μl), with dose 12.5 mg/kg, was injected daily by intravenous injec- tion for 7 days.

The CIA model was established according to literature46. Female Lewis rats were given intradermal injections of bovine type II collagen with the Freund’s adjuvant (emulsion of two reagents at volume ratio = 1:1), one site at the tail base (0.1 ml) and two sites at the back (0.2 ml per site) on day 0 and day 7. The onset of arthritis occurred on day 13, and the arthritic rats were evenly divided into model and treatment groups at random. The effect of cationic polymers was evaluated in both early stage and established stage of RA. For the early stage RA progression, the treatment began at day 13 with five groups animal. The rats without disease were applied as the normal control (n = 5). The CIA rats without treatment were the model control (n = 10). The other three treatment groups, 10 rats/group, were the treatment by PDMA (12.5 mg/kg) and cNP (12.5 and 25 mg/kg). For the established stage treatment, the normal and model groups were the same as above, whereas for the treatment group, we selected a dose of 25 mg/kg cNP (n = 5) to evaluate its efficacy.

Joint swelling measurements and clinical scores. The swelling and clinical scores of joint were evaluated daily from the onset of arthritis (day 1 for mice and day 13 for rats), until the animals were sacri- ficed at day 8 and rats sacrificed at day 29 for mice and rats, respectively, to assess the severity of hindpaw, digital and wrist joints of sacrificed animals as well as their heart, liver, spleen, lung and kidney were fixed in 10% buffered formalin and then tissues were incubated in decalci-fying solution (4% hydrochloric acid in 4% formaldehyde) at room temperature for 7 days for decalcification. After paraffinization, microtome (Leica) slices of 2 μm were cut and stained. The slides were stained with haema-toxylin and eosin, respectively, and the inflammatory cell accumulation in synovial tissues, bone and cartilage was evaluated by Vectra Automated Quantitative Pathology Imaging System (PerkinElmer). For immunohistochemical staining, after deparaffinization the slices were subjected to antigen recovery in 0.1 M sodium citrate buffer at 125 °C for 30 min, followed by 3 min at 90 °C, and then subjected to the endogenous peroxidase inactivation by covering tissue with 3% hydrogen peroxide for 5 min. After blocking non-specific binding sites with 10% goat serum in PBS, the slices were incubated with different biotinylated monoclonal antibody or polyclonal antibody with a dilution of 1:100, respectively, at 4 °C overnight. Then the slices were incubated with 1:100 diluted HRP-conjugated secondary antibody with a dilution rate of 1:800 at 37 °C for 1 h. Sections were developed using the DAB substrate and then counterstained with haematoxylin. The biotinylated monoclonal antibody or polyclonal antibody used were anti-TNF α antibody, anti-IL 6 antibody, and anti-MMP-3 antibody. The images were captured and analyzed by Vectra Automated Quantitative Pathology Imaging System. The positive results (brown staining) were evaluated using Nuance 3.0.2 and inform 2.1.1 software.

For evaluation of acute toxicity of cationic materials, the lungs of normal rats and treated rats after i.v. injection of 25 mg/kg of cNP were photographed at 24 h intervals. The lung of the rats treated with 75 mg/kg of cNP was photographed immediately after it died within 10 min. The lungs of sacrificed rats were then embedded in O.C.T. compound, quickly frozen at –80 °C and then sectioned at 6 μm. H&E histological examination of the lungs was performed by a trained pathologist (SML) using a scoring system as previously described47: synovial cell lining hyperplasia (0–3); mononuclear cell infiltration (0–3); mononuclear cell infiltration and polymorphonuclear leukocyte infiltration in periarticular soft tissue (0–3); cellular infiltration and bone erosion at distal tibia (0–3); and cellular infiltration of cartilage (0–2). The histology score of each animal joint was the sum of all the histopathologic feature scores.

Rotational cage test study. At the end of treatment (day 29), the move ability of normal, model and cNP established stage treated rats (n = 3 per group) were placed on a programmable, motorized wheel apparatus (21 cm diameter, 40 cm length, made in South China University of Technology, Guangzhou, China), and the movement was recorded on a video for 10 min. The distance traveled on a fixed turntable and the total distance were measured.

Examination of liver and renal function markers. Peripheral blood sample of rats from different groups were taken from the eye socket at day 13, 29, and 60 after first collagen II injection. After 2 h, the samples were centrifuged at 8000 × g for 10 min. The supernatant was obtained, and the protein concentration was measured. The volume of serum was then added to 800 μl of cell-free serum and stored at –80 °C for analysis. The levels of ALP, ALT, AST, creatinine, urea, and uric acid were measured by KingMed Diagnostic Company.
**Real-time PCR.** At day 29, the shinbones of sacrificed rats were removed and homogenized in TRIzol™ reagent with a homogenizer (T 18 digital ULTRA-TURRAX, IKA) according to the reference.19 Briefly, total RNA was extracted using Rneasy-universal Tissue Kit, then 2 μg of RNA was reverse-transcribed into cDNA in a total volume of 20 μL using PrimeScript RT reagent Kit With gDNA Eraser and oligo (dT). The cDNA levels were measured by SYBR green real-time in the Lightcycler (Applied Biosystems QPCR) and were normalized with that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The relative mRNA levels of cytokines and MMP-3 in normal group were set as 1. The sequences of the primers were as follows: qTNF-α AP: 5′-TGTACCAAGATGTTGAAGCT-3′; qTNF-α AP: 5′-TCGAGGAAATGAGGAAG-3′; qIL-6 AP: 5′-GGTCTTACACTGTCGTCAGA-3′; qIL-6 AP: 5′-GGTCTTACACTGTCGTCAGA-3′; qMMP-3 AP: 5′-CCGAGCTGTCGTCGTCATC-3′; qMMP-3 AP: 5′-GAGGAGCAACCGAGAATGAG-3′.

**Biodistribution of cationic materials.** At day 30 after the first immunization, the untreated CIA rats and normal rats were anaesthetized (1.5% isoflurane, 0.5 ml/min oxygen) and the Alexa Fluor® 750 labeled cationic materials were intravenously injected. The biodistribution of the materials in four groups, including PDMA-treated normal group, PDMA-treated model group, cNP-treated normal group, and cNP-treated model group, was monitored by NIRF imaging using an in vivo imaging scanner (Carestream FX PRO) at different time points during 24 h. At 4 and 24 h, one from each group was sacrificed for dissection. The joints, thymus, heart, lung, liver, kidney, pancreas, spleen, and bladder were taken out for ex vivo NIRF imaging and their mean NIRF intensity was calculated by Bruker MI software.

**Statistical analysis.** Statistical significance of survival curve of rats was calculated by log-rank (Mantel–Cox) test using GraphPad Prism 6.0, and statistical analysis of other experimental data was performed by one-way ANOVA with LSD post-test using IBM SPSS Statistic 22.

**Data availability** All data supporting the findings of the current study are available in the article and its Supplementary Information. Additional relevant data are available from the corresponding author upon reasonable request.

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**Author contributions**
H.L. conducted the main experiments; B.P. assisted with DNA binding and cell inhibition assays; C.D. assisted with biological experiments; I.L. designed and supervised all the experiments and wrote manuscript; J.M. conducted medical imaging; I.S. supplied imaging and analysis; S.W. and X.W. supplied RA samples; H.X. and X.G. provided discussion on RA; K.W.L. and H-Q.M. supervised and wrote manuscript; Y.C. designed materials, supervised and wrote manuscript.

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