Biocompatible Amino-modified Tantalum Nanoparticles with Catalase Activity for Sustained Intra-articular Reactive Oxygen Species Scavenging and Alleviation of MIA induced Osteoarthritis

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Abstract

Background

Osteoarthritis (OA), which involves the dysfunction of articular cartilage, is the most common form of joint disease that results in arthralgia, joint deformation and limited mobility in patients. Recent studies highlighted the vital role of oxidative stress and reactive oxygen species (ROS) during progression of OA. Therefore, attenuating oxidative stress and reducing ROS generation in articular joints represent reasonable strategies for the treatment of OA. However, in addition to instability of current antioxidants caused by fluctuation in osteoarthritic physicochemical microenvironment, poor biocompatibility and short articular joint retention also seriously hindered their clinical application.

Results

Considering the above-mentioned, the present study provided high biocompatible small positively charged tantalum nanoparticles (Ta-NH$_2$ NPs) with sustained intra-articular catalase activity. Our in vitro results showed that Ta-NH$_2$ NPs had good biocompatibility and stability, and could protect viability and hyaline-like phenotype in chondrocyte under H$_2$O$_2$ challenge. In addition, the in vivo biodistribution data demonstrated sustained retention of Ta-NH$_2$ NPs in the joint cavity, particularly in articular cartilage with unnoticed organ toxicity and abnormity in hemogram and blood biochemistry analyses. Finally, compared with catalase (CAT), Ta-NH$_2$ NPs exhibited long-term therapeutic effect in monosodium iodoacetate (MIA) induced OA model.

Conclusion

This study explored the potential of Ta-NH$_2$ NPs as effective ROS scavenging agent for intra-articular injection, and offered a novel strategy to achieve sustained ROS suppression using biocompatible Ta-based nano-medicine in oxidative stress related diseases.

Introduction

Osteoarthritis (OA), which involves the dysfunction of adult articular cartilage, is the most common form of joint disease with manifestations of chronic joint inflammation, and may result in arthralgia, joint deformation and limited mobility in patients [1]. OA is the leading cause of long-term disability in adults, and the prevalence of OA is estimated to reach 40% [2]. OA is characterized by morphological, biochemical, molecular and biomechanical changes of both cells and extracellular matrix (ECM) in articular cartilage, synovium, and subchondral bone. Although the mechanisms of OA initiation and progression are yet to be understood, recent studies have highlighted the vital role of oxidative stress and reactive oxygen species (ROS) in mitochondrial dysfunction [3], chondrocyte senescence [4], matrix
synthesis [5], synovial inflammation [6], and subchondral bone dysfunction during progression of OA. Oxidative stress has been defined as a disturbance in the balance between the production of ROS and antioxidant defenses, which results in macromolecular damage and disruption of redox signaling and control [7]. Oxidative stress amplifies inflammatory responses and exacerbates cartilage breakdown [8]. Whereas attenuating the oxidative stress level by antioxidants drugs or natural antioxidants could effectively decrease the progress of OA in animal models [9]. Therefore, attenuating oxidative stress and reducing ROS generation in articular joints represent reasonable strategies for the treatment of osteoarthritis.

Currently, antioxidant supplementations [9], mediators of various ROS pathways [7], and free radical scavengers have been successively utilized to target oxidative stress in the pathogenesis of OA. Among them, the commonly used antioxidants are mainly divided into macromolecular and small molecular types. However, systemic application of these antioxidants is easily related to poor safety, pharmacodynamics, and bioavailability [10]. ROS scavenging therapy for OA is thus administered by intra-articular injection. However, in addition to instability of these antioxidant caused by fluctuation in osteoarthritic physicochemical microenvironment, small molecules are rapidly cleared from the joint within hours by the synovial vasculature, and large molecules are cleared by the synovial lymphatic vessels within days [11]. Therefore, high-dosage injections are often required to achieve the desired effect [12]. To overcome these shortcomings and prolong the action of antioxidant for further clinical application, inorganic metal nanoenzyme including ultrafine copper oxide [13] and Fe-curcumin nanozyme [14] were developed. These reactive metal-based nanomaterials have displayed good bioavailability and excellent therapeutic efficiency at very low concentration (∼ nM/mL) to treat oxidative stress-related diseases in animal model. However, the potential disadvantages such as ionization in body fluid [15] and uncertainty of long-term metal cumulation [16, 17] still impede its clinical application.

Tantalum (Ta) is an inert metal with strong corrosion resistance and excellent biocompatibility [18]. Ta-based implantations are widely used in joint replacement and bone defect repair [19]. Unlike cobalt and titanium, Ta-based implants have been reported to maintain an excellent long-term biocompatibility [18]. In addition to the application in tissue engineering, tantalum nanoparticles (Ta NPs) have also gained reasonable attention in early diagnosis and photothermal therapy of malignant tumor due to its high biocompatibility. For instance, recent studies have demonstrated that tantalum oxide nanoparticles are an excellent contrast agent for computed tomography (CT) [20]. In addition, 2D tantalum sulfide [21] and tantalum carbide nanosheets [22] have also been explored as light-induced therapeutic agents for tumor diagnosis and therapy. Interestingly, studies also suggested tantalum-coated surfaces could effectively alleviate oxidative stress induced by a high-glucose environment in a diabetes model [23], indicating the similar properties to catalase of Ta-NPs [24]. The aforementioned properties endow Ta-NPs with the ability to effectively alleviate oxidative stress by eliminating H₂O₂-induced ROS, and make it possible for its application in the treatment of OA.

Based on this, the present study designed small-diameter Ta-NPs with amino-modification for OA-related oxidative stress modulation. The surface of Ta-NPs was modified by amino group (Ta-NH₂ NPs) with
Results And Discussion

Ta-NH$_2$ NPs synthesis and characterization.

Pristine Ta NPs with high density have poor colloidal stability in common aqueous [24]. To improve the colloidal stability of Ta NPs and increase their retention in negatively charged articular cartilage extracellular matrix (ECM) [25], the surface of Ta NPs was modified with amino group. Ta-NH$_2$ NPs were synthesized by a simple and efficient silane-coupling approach, which was widely used for modification of the surface of inorganic NPs [26]. In detail, the commercial raw Ta NPs were dispersed in ethanol and then centrifuged to separate large-sized NPs. Amino groups were coated on the surface of Ta NPs by refluxing reaction with (3-Aminopropyl) trimethoxysilane in ethanol. From scanning electron microscopy (SEM) (Fig. 2A and B) images, Ta-NH$_2$ NPs exhibited uniform monodispersed compared with Ta NPs. Meanwhile, in consistent with previous report [24], as shown in the TEM image (supplementary Fig. 1A, the Ta NPs and Ta-NH$_2$ NPs were observed with irregular elliptical morphology. HR-TEM elemental mapping images (supplementary Fig. 1B) indicated that Ta, O, and N elements were uniformly distributed in Ta-NH$_2$ NPs. Whereas only elemental Ta and O emerged in Ta NPs, verifying the successful modification of amino group on Ta NPs surface. As shown in Fig. 2C, the hydrodynamic sizes of Ta NPs and Ta-NH$_2$ NPs determined by dynamic light scattering (DLS) were 116.73 ± 0.31 and 265.10 ± 9.45 nm with a narrow PDI (0.18 ± 0.01 vs. 0.33 ± 0.05), respectively. Meanwhile, the zeta potential changed from negative (Ta NPs, -36.63 ± 1.31 mV) to positive charge (Ta-NH$_2$ NPs, 32.27 ± 0.51 mV) (Fig. 2D), which could be explained by the modification of Ta NPs with amino group ligands spreading out in solutions. As shown in the XRD patterns (Fig. 2E), Ta-NH$_2$ NPs maintained all the characteristic peaks of the Ta NPs at high angles of 30° – 80°, which indicated that the modification of amino group on Ta NPs surface did not decrease the crystalline purity of Ta NPs. In addition, from FT-IR spectra (Fig. 2F), the Ta-NH$_2$ NPs appeared new characteristic bands around 3300 cm$^{-1}$ of -NH$_2$, confirming that amino group had been successfully bonded on the surface of Ta NPs. In addition, the chemical valence of Ta$_2$O$_5$ and metallic Ta could be detected at 28 eV (Ta4f7/2) and 26 eV (Ta4f5/2) from the XPS spectra (Fig. 2G), which could be...
attributed to partial oxidation on the surface of Ta NPs and turned into a more stable form [27]. The oxygenic groups on metal surface were beneficial to the reaction of silane with surface oxygenic groups of Ta NPs [28]. Meanwhile, the chemical valence of Si and N elements also could be observed at 100 eV (Si2p) and 404 eV (N1s) from the XPS spectra (supplementary Fig. 2), respectively, further confirming the successful modification of (3-Aminopropyl) trimethoxysilane on Ta NPs surface. As shown in Fig. 2H, Ta-NH₂ NPs exhibited stable dispersion in H₂O for 5 days. Furthermore, no obvious hydrodynamic particle size change was observed in Ta-NH₂ NPs after incubation in H₂O for 5 days, indicating a good colloidal stability (Fig. 2I). Taken together, our data demonstrated that amino groups had been successfully decorated on the surface of Ta NPs with improved colloidal stability.

H₂O₂ scavenging activity of Ta-NH₂ NPs.

H₂O₂, the representative ROS [29] was selected to investigate the ROS scavenging activity of Ta-NH₂ NPs in vitro. As shown in Fig. 3A, 1 mM H₂O₂ was reacted with different concentration of Ta-NH₂ NPs at 37 °C for 1 h. Ta-NH₂ NPs exhibited ROS scavenging activity in a concentration-dependent manner. Approximately 35% of the H₂O₂ was decomposed by 50 µg/mL Ta-NH₂ NPs, and almost 40% H₂O₂ could be scavenging in the concentration 100 µg/mL with excellent pH and temperature stabilities (Fig. 3B and C). Compared with the working concentrations of reported metal nanoparticles, recently reported ultrasmall copper oxide [13] and manganese dioxide nanoparticles [30] might manifest better catalytic efficiency than Ta-NH₂ NPs. However, these metal nanomaterials might be ionized by body fluid [15] to generate free metal ions, which further led to element imbalance or even metal poisoning [16, 17]. In addition, compared with inert metal such as cerium and gold nanoparticles [31], Ta-NH₂ NPs showed stronger H₂O₂ decomposition. Compared with traditional antioxidants, the stability analysis indicated that the H₂O₂ scavenging of Ta-NH₂ NPs was not influenced by pH or temperature [32]. It is well known that the activities of ROS scavenging in traditional biological enzymes [33] were affected by the microenvironment of joint cavity, and this drawback might affect the therapeutic outcome due to the fluctuation in physical and chemical properties of osteoarthritic joints.

The biocompatibility of Ta-NH₂ NPs in vitro.

The biocompatibility of Ta-based materials has been widely validated in orthopedic implants [18]. In the present study, our in vitro biocompatibility analysis suggested that Ta-NH₂ NPs also shared the same characteristics in safety. The CCK-8 assay results showed no significant cytotoxicity at test concentration after 24 h and 48 h co-culture (Fig. 3D). But slightly decreased chondrocyte viability was noticed when concentration reached 200 µg/mL. Chondrocytes after cocultured with Ta-NH₂ NPs did not manifest obvious morphology change. In addition, Western blot (Fig. 3E and G) results showed that no significant changes in protein levels of COL-II, SOX9, ACAN, MMP13, RUNX2, and ADAMTS5 post 24h treatment. Furthermore, crystal violet staining, alcian blue staining, and immunofluorescent staining of COL-II and ACAN results indicated Ta-NPs treatment did not affect the deposition of cartilaginous ECM(Fig. 3I).
Ta-NH$_2$ NPs protects viability and hyaline-like phenotype in chondrocyte under oxidative stress in vitro.

During the progression of OA, iNOS from OA-affected cartilage may contribute to the inflammation and pathogenesis of cartilage destruction [7]. Chondrocytes isolated from unhealthy OA cartilage showed over expression of iNOS mainly in the superficial zone [34]. Expression of iNOS could reflect the degree of oxidative stress [7]. To investigate the inhibitory effect of Ta-NH$_2$ NPs on iNOS and ROS production in chondrocyte under oxidative stress, we pre-treated cells with Ta-NH$_2$ NPs (100 µg/mL) or catalase (CAT, 100 µg/mL) for 1 h. Cells were then challenged with H$_2$O$_2$ for 24 h (400 µM). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) staining indicated significant increase in intra-cellular ROS level in H$_2$O$_2$ treated group (Fig. 4A), which was inhibited by either Ta-NH$_2$ NPs or CAT. In agreement with previous studies, our data showed significant increase in iNOS expression post H$_2$O$_2$ challenge. While pre-treatment with Ta-NH$_2$ NPs, but not CAT reversed the iNOS level. In addition, live and dead staining data suggested, Ta-NH$_2$ NPs or CAT pre-treatment successfully protected chondrocyte viability via inhibiting intra-cellular ROS production. These data indicated that although Ta-NH$_2$ NPs and CAT showed similar protective effect under oxidative stress, different mechanism might be involved. In the aspect of phenotypic alternation, our immunofluorescence staining and Western blot results suggested that H$_2$O$_2$ significantly decreased the hyaline-like phenotype in chondrocytes, while increased fibrotic (COL-I) and hypertrophic (COL-X, RUNX-2, and MMP-13) markers [35, 36]. It is worth mentioning that fibrosis and hypertrophy are important pathological change in osteoarthritic cartilage [7, 8]. Previous studies have revealed that reduction of oxidative stress attenuated fibrosis and hypertrophy indexes effectively in tissue-organ level, including liver [37], kidney [38], and heart [39]. Similar to CAT, Ta-NH$_2$ NPs inhibited the increment in protein levels of COL-I, RUNX-2, and MMP-13 (Fig. 4C), and maintained the hyaline-like phenotype (ACAN and COL-II) in H$_2$O$_2$ treated chondrocytes. Compared with CAT group, it is worth noticing that Ta-NH$_2$ NPs group showed more significant increase in ACAN and COL-II, while decrease in ADAMTS-5 protein. The observation that Ta-NH$_2$ NPs was more capable of restoring the balance between catabolism and anabolism might be attributed to the inhibitory ability of Ta-NH$_2$ NPs in iNOS expression. Previous study suggested that NO promoted degradation of ECM by enhancing the activity of matrix metalloproteinase (MMPs) that subsequently led to joint destruction [40].

In vivo biodistribution and biocompatibility assessment of Ta-NH$_2$ NPs.

Currently, drug delivery by intra-articular injection suffers from short retention in the joint cavity [10]. Small molecules are rapidly cleared from the joint cavity within hours via synovial vasculature, and macromolecules within days via synovial lymphatics [11]. In order to achieve stable pharmacokinetics, increased dosage or repetitive injection are often necessary [12]. In addition to biocompatibility, the ideal therapeutic antioxidant should also have high efficiency and long-term effect. In the present study, the positive charged NPs were designed to prolong the retention of Ta-NPs in articular cartilage. As shown in Fig. 5, after intra-articular injection of Cy5.5 labeled Ta-NH$_2$ NPs (100 µg/mL) and CAT (100 µL), in vivo fluorescence imaging indicated gradually decreased concentration of both antioxidants at 1, 3, 7, 14 and 28 d. Whereas the Cy5.5 dye alone showed rapid decrease in fluorescence intensity after 1 h post
injection (supplementary Fig. 5). Compared with CAT group (at 3 d), significantly longer joint retention (at 28 d) of Ta-NH$_2$ NPs was noticed. In addition, further analysis in dissected knee joints confirmed that Cy5.5 labeled Ta-NH$_2$ NPs, but not Cy5.5 labeled CAT (supplementary Fig. 5) was still detectable after 28 d post injection in femoral articular cartilage (Fig, 5B and C). Agreed with previous study, these data confirmed the positively charged Ta NPs we prepared was able to stay in the joint cavity, particularly in articular cartilage [41]. From the imaging of the dissected femoral condyle, sustained fluorescence over 28 d observational period outlined the shape of trochlear cartilage, indicated that the Ta-NPs after amino modification could be adsorbed in the surface of the articular cartilage.

To further explore the biocompatibility and potential organ toxicity of Ta-NH$_2$ NPs, we analyzed the biodistribution in main organs. Liver, heart, spleen, lung, kidney and brain from rat post intra-articular injection of Cy5.5 labeled Ta-NH$_2$ NPs were tested. No hemorrhage, atrophy, or necrosis was found in the analyzed organs. At the macroscopic level, Ta-NH$_2$ NPs mainly accumulated in the liver, spleen and kidney. Among them, the fluorescence intensity in liver and kidney reached the peak at day 3 post injection and decayed by day 7. However, the fluorescence intensity in liver and kidney increased again at day 14. This observation suggested that Ta-NH$_2$ NPs in the organ might be gradually metabolized, but required more than 28 days. To evaluate the organ toxicity of Ta-NH$_2$ NPs, histological analysis was employed. H&E staining of main organs indicated no necrosis, congestion, or hemorrhage in the heart, liver, spleen, and lung at 1, 3, 7, 14 and 28 days (supplementary Fig. 6) after single dose intra-articular injection of Ta NPs. Moreover, no distinguishable inflammatory, lesion or tissue damage was observed in the glomerulus, tubules, collecting ducts, and urethra, illustrating the excellent biocompatibility of Ta-NH$_2$ NPs. To further explore the functional changes of these organs, the hemogram and blood biochemistry analyses were performed. We selected AST and ALT, CRE and BUN to reflect the liver and renal functions, respectively. Compared with control group, our data showed no significant alternation in the size, number, and composition of blood cells, and in AST, ALT, BUN, and CRE levels.

In vivo therapeutic effect of Ta-NH$_2$ NPs in MIA induced osteoarthritic model.

As shown in Fig. 7A, the rat osteoarthritis model was induced by sodium iodoacetate MIA injection, followed by the corresponding antioxidant injection. At 8 w post injection, compared with that in the sham operation group, the micro-CT scanning indicated obvious bone defects in the patella and femoral condyle, and alternation in subchondral bone structure in vehicle group (Fig. 7B). The subchondral bone structure was significantly improved by Ta-NH$_2$ NPs or CAT injection ($P<0.05$, Fig. 7C). And compared with CAT group, Ta-NH$_2$ NPs showed superior therapeutic effect in patellar bone tissue restoration.

In order to verify the long-term anti-oxidative stress effect of Ta-NH$_2$ NPs post single intra-articular injection, iNOS expression was detected in articular cartilage, subchondral bone and synovium during the entire process. The expression of iNOS in articular cartilage was significantly increased, and the expression reached the peak at the 8th week after MIA injection (Fig. 7D). Antioxidant treatment decreased the expression of iNOS, particularly in Ta-NH$_2$ NPs treated group. In addition, Ta-NH$_2$ NPs, but
not CAT showed prolonged inhibition of iNOS expression at the 8th week after MIA injection (Fig. 7D). Cartilage is the main source of NO in OA [42], and iNOS expression is more enhanced in chondrocytes compared with synovial cells from patients with OA [42]. Furthermore, in chondrocytes isolated from osteoarthritic cartilage, the over expression of iNOS was mainly concentrated in the superficial region [34], whereas chondrocytes isolated from patients without OA did not express iNOS. These observations were in lined with our data, in which we showed iNOS expression was mainly confined to articular cartilage, especially in the superficial zone. The cartilage-targeting ability of Ta-NH$_2$ NPs might act through direct oxidation resistance in the ECM, or reduce intracellular iNOS synthesis via phagocytosis. The iNOS expression in synovium tissue was similar to cartilage, whereas no significant difference in iNOS expression in subchondral bone was noticed.

The synovium is a thin connective tissue that attaches to the joints, its inflammation is mediated by activation of mitochondrial dysfunction [6], cytokines, and metabolites in synovial cells. The intercommunication between chondrocytes and synovial cells is thought to be beneficial to joint homeostasis [43]. Once synovitis is activated, cartilage undergoes subsequent adverse changes. In addition to the immunohistochemical staining of iNOS, we further observed synovial inflammation and angiogenesis by H&E staining. It was not difficult to notice the MIA-induced synovial inflammation (Fig. 7D). In vehicle group, obvious hyperplasia and inflammatory cell infiltration were seen in synovium at 4 weeks post intra-articular injection. In addition, vessel hyperplasia was also noticed at 8 weeks. The inflammation of the synovium was alleviated after both anti-oxidative treatments. Notably, synovitis score indicated synovial inflammation in the Ta-NH$_2$ NPs group was significantly lower than that in the CAT group at 8 weeks post injection (Fig. 7E).

The OARSI scores were shown in Fig. 8. H&E and safranin-o-fast green staining showed that the vehicle group presented obvious typical osteoarthritic features such as surface irregularity, decreased expression of glycosaminoglycans, and cartilage defects. Compared with vehicle group, both antioxidants attenuated the OARSI score in femur, and significantly decreased the OARSI score in tibia. Agreed with our previous data, Ta-NH$_2$ NPs exhibited excellent long-term therapeutic effect as evidence by more homogeneous glycosaminoglyc and cell arrangement (P<0.05 when compared with CAT group).

**Conclusion**

To overcome the disadvantages of current antioxidative strategy for OA, the present study designed and prepared small positively charged Ta nanoparticles with sustained intra-articular catalase activity. The *in vitro* results showed that our designed Ta-NH$_2$ NPs had good biocompatibility and stability, and protected viability and hyaline-like phenotype in chondrocyte under oxidative stress. Our hypothesis was agreed with *in vivo* biodistribution data that Ta-NH$_2$ NPs showed sustained retention in the joint cavity, particularly in articular cartilage with excellent *in vivo* biocompatibility. Finally, Ta-NH$_2$ NPs exhibited long-term antioxidant stress and therapeutic effects in MIA-induced OA model.
Materials And Methods

Surface amino modification of Ta NPs.

The surface of Ta NPs (Sigma Aldrich, St. Louis, USA) was modified with amino group using the silane-coupling method. Briefly, Ta NPs (1 g) was dispersed in ethanol (10 mL) under tip ultrasound for 20 min, followed by centrifugation (1 000 rpm, 5 min) to remove large sized NPs. The concentration of collected solution was determined with oven drying method and diluted to 1 mg/mL with ethanol. (3-Aminopropyl)trimethoxysilane (100 µL, Aladdin, Shanghai, China) was added into Ta NPs (20 mL) and stirred for another 3 h at 70 °C. Finally, Ta-NH$_2$ NPs were obtained by centrifugation (12 000 g, 5 min) and washed with deionized water to remove the free (3-Aminopropyl)trimethoxysilane for three times.

Characterization of Ta and Ta-NH$_2$ NPs.

The morphology of Ta NPs and Ta-NH$_2$ NPs were observed using Hitachi S-3400NIISEM (Hitachi, Japan) at 2 kV accelerating voltage. Transmission electron microscope (TEM) and element mapping images were conducted by JEM 2100F (JEOL, Japan). The hydrodynamic sizes, particle dispersity index (PDI), and zeta-potential of Ta-NH$_2$ NPs were analyzed with the Malvern Zetasizer Nano ZS instrument (Malvern, UK). Fourier transforms infrared (FT-IR) spectra of Ta-NH$_2$ NPs was measured using Nicolet IS 10 spectrometer (Nicolet, USA). X-ray diffraction (XRD) of Ta-NH$_2$ NPs was detected by an X-ray diffractometer D8 ADVANCE (Bucker, Germany) with the 2-Thera range from 10° to 90°. X-ray photoelectron spectroscopy (XPS) of Ta-NH$_2$ NPs was determined by an X-ray photoelectron spectrometer 250Xi (Thermo Fisher, USA).

H$_2$O$_2$ scavenging activity assay.

H$_2$O$_2$ scavenging activity of Ta-NH$_2$ NPs was tested by the Hydrogen Peroxide Detection Kit (Beyotime, Beijing, China). Ta-NH$_2$ NPs with different concentrations (0, 10, 20, 50, and 100 µg/mL) were incubated with 1 mM H$_2$O$_2$ at 37°C for 1 h, respectively. After reaction, the concentration of remained H$_2$O$_2$ was determined according to the manufacturer’s instructions, and the scavenging capacity was calculated. For assessment of Ta-NH$_2$ NPs with stable H$_2$O$_2$ scavenging activity, Ta-NH$_2$ NPs (100 µg/mL) were incubated with 1 mM H$_2$O$_2$ under different temperature (25, 37, 50, and 60 °C) and pH (2, 4, 6, 7, 8, and 10), respectively.

Isolation of chondrocyte and cell culture.

All animal experiments were performed in accordance with the guidelines approved by the Laboratory Animal Welfare and Ethics Committee of Army Medical University (Chongqing, China). The methods of isolation chondrocytes were in accordance with the previously published articles[44]. Male Sprague-Dawley (SD) rats were asphyxia by CO$_2$, and knee joint was dissected. The cartilage was cut into pieces and digested with type II collagenase for 12 h. The obtained chondrocytes were cultured with Dulbecco’s
modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 U/mL penicillin at 37°C in 5% CO₂ incubator.

In vitro cytotoxicity evaluation of Ta-NH₂ NPs.

The cytotoxicity of Ta-NH₂ NPs was determined by the CCK-8 assay in vitro. Briefly, chondrocytes (1 × 10⁴ per well) were seeded into 96-well culture plates and incubated at 37°C in an 5% CO₂ incubator for 24 h. Different concentrations of Ta-NH₂ NPs (0, 5, 10, 20, 50, 100, 200, and 500 µg/mL) were added to each well and incubated for another 24 h or 48 h. The cell viability was then quantified by measuring the absorbance value at 450 nm with microplate reader.

Detection of intracellular ROS.

Chondrocytes (1 × 10⁵ per dish) were seeded into confocal petri dish and pre-treated with Ta-NH₂ NPs (100 µg/mL) for 2 h. 400 µM H₂O₂ was then added into petri dish and cells were incubated for another 24 h. After washing with PBS, cells were stained with DCFH-DA probes following the manufacturer's instructions and subjected to flow-cytometry detection.

Immunofluorescence Staining.

The above-described cells were washed with PBS, and fixed with 4% paraformaldehyde, followed by blockage with Quick Blocking buffer. Next, the cells were incubated with primary antibody (COL-I, COL-X, COL-II, SOX-9, ACAN, MMP-13, ADAMTS-5, and RUNX-2) and then with secondary antibody. Nuclear was stained with DAPI. The fluorescence images were observed with laser scanning confocal microscope, and the relative fluorescence intensity was analyzed with ImageJ (version 1.52p).

Western blot analysis.

The total proteins were isolated from above-described cells using RIPA lysis, and were quantified using BCA protein kit according to the manufacturer's instructions. The protein were separated by SDS PAGE and subsequently transferred to PVDF membrane. Next, the cell lysis was incubated with primary antibody (COL-I, COL-X, COL-II, SOX-9, ACAN, MMP-13, ADAMTS-5, and RUNX-2), followed by secondary antibody incubation. Finally, the membranes were visualized using ultrasensitive ECL and the intensity of blots was quantified with Image Lab software (version 3.0).

Intravital fluorescence imaging.

To determine the retention time of Ta-NH₂ NPs in the articular joint, male SD rats (250–300 g) were intra-articular injected with 100 µL of Ta-NH₂ NPs-CY5.5 (100 µg/mL). The fluorescence images were captured at various time points (1, 3, 7, 14, and 28 d) using In Vivo Imaging System Pearl Trilogy (LI-COR, USA). The knee joint, femur condyles, and major organs (heart, liver, spleen, lung, kidney, and brain) were
harvested for *ex vivo* NIR imaging. The relative fluorescence intensities were analyzed using Image Studio (version 5.2).

In vivo toxicity evaluation of Ta-NH$_2$ NPs.

Blood samples were harvested at day 1 and 28 after intra-articular injection of 100 µL of Ta-NH$_2$ NPs. The serum biochemistry tests included two important indicators of hepatic function: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and two indicators of kidney function: blood urea nitrogen (BUN) and creatinine (CRE). Furthermore, harvested major organs (liver, heart, spleen, lung, kidney, and brain) were subjected to hematoxylin and eosin (H&E) staining and histological analysis.

MIA-induced OA Model and Treatment with Ta-NH$_2$ NPs.

The rat OA model was induced by intra-articular injection 20 µL of MIA (2 mg/mL) for 2 weeks. Rats were randomly divided into 4 groups (n = 8): Control group (Intra-articular injection of PBS), MIA group (Intra-articular injection of MIA), MIA + Ta-NH$_2$ NPs group (Intra-articular injection of MIA, followed by Ta-NH$_2$ NPs treatment), and MIA + CAT group (Intra-articular injection of MIA, followed by CAT treatment). Rats were sacrificed after treatment with Ta-NH$_2$ NPs or CAT for 4 or 8 weeks. The knee joints were collected and fixed in 4% paraformaldehyde.

Micro CT imaging.

The knee joints were scanned with micro-CT (viva CT-40, ScancoMedical AG, Switzerland). Image acquisition was performed with the condition of 45 kV and 177 µA in high-resolution scans (10.5 µm voxel resolution). Two-dimensional images were used to generate three-dimensional reconstructions. The epiphysis of the tibial subchondral bone was manually chosen as the region of interest during three-dimensional analysis (micro-CT Evolution Program V6.5 software).

Histological analysis.

The knee joints were decalcified in 10 wt% ethylenediamine tetraacetic acid (EDTA) solution for 4 weeks at 25°C. To observe the degeneration of knee articular cartilage, synovial inflammation and corresponding inducible nitric oxide synthases (iNOS) changes, H&E, Safranin-o-fast green staining, and immune-histochemical staining against iNOS were performed respectively. Three independent experts were asked to perform double-blind scoring according to Osteoarthritis Research Society International (OARSI) scoring system.

*Statistical analysis.*

Graphical results were displayed as mean ± SD by using GraphPad prism software (version 7.0). All data were assessed for normality using the Kolmogorov-Smirnov test and for homoscedasticity using the F-test. The statistical significance difference between two groups was compared by independent-sample t-
test for parametric data, and by Mann-Whitney test for nonparametric data. The Welch's correction was applied for variables with unequal variance. The statistical significance differences between vehicle and other treatment groups were determined by One-way ANOVA test and Fisher's LSD post-test for parametric data, and by Kruskal Wallis test and Dunn's multiple comparisons post-test for nonparametric data. In all cases, statistical significance was defined with $P < 0.05$.

**Abbreviations**


**Declarations**

**Author contributions**

Experiment Section: Designed and performed by Y. J., T. L., X.S., and J. C.. Supervised by X. G. and L.Y.. Experimental data were processed by Y. J., J. Y. and X. W.. Manuscript Section: Written by Y. J., G. C., G. D., R. L., and C. Y.. All figures were drawn by Y. J. and T. L.. Funding Section: Provided by L. Y. and X. G.. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Data availability statement**

All datasets used and analyzed in this article can be obtained from the corresponding author.

**Ethics approval and consent to participate**

All female SD rats were provided by the Animal Center of Army Medical University. The study in vivo was performed under protocols approved by the Animal Management Rules of the Ministry of Health of the
People's Republic of China (document no. 55, 2001) and the examination and approval of the Laboratory Animal and Welfare Ethics Committee of the Third Military Medical University.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest.

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**References**


**Figures**
Figure 1

Schematic diagram of the synthesis of Ta-NH$_2$ NPs and the treatment of osteoarthritis. Negatively charged NPs were obtained by adding amino groups to the surface of Ta, and injected into the joint cavity of OA rats. Ta-NH$_2$ NPs can alleviate OA progression by attenuating oxidative stress.
Figure 2

Morphological and compositional characterizations of Ta-NH2 NPs. (A) SEM image of Ta NPs and (B) SEM image of Ta-NH2 NPs. (C) Hydrodynamic diameters and (D) Zeta potential of Ta NPs and Ta-NH2 NPs measured by DLS. (E) XRD patterns and (F) FT-IR spectra of Ta NPs and Ta-NH2 NPs. (G) XPS spectra of Ta-NH2 NPs, (H) Representative digital photographs of Ta NPs and Ta-NH2 NPs at 0 d and 5 d. (I) Hydrodynamic diameters of Ta-NH2 NPs measured by DLS at 1-5 d.
**Figure 3**

H$_2$O$_2$ scavenging activity and *in vitro* biocompatibility of Ta-NH$_2$ NPs. (A) Concentration dependent H$_2$O$_2$ scavenging activity of Ta-NH$_2$ NPs. (B) pH and (C) temperature stabilities of H$_2$O$_2$ scavenging activity in Ta-NH$_2$ NPs. (D) Chondrocyte viability under different Ta-NH$_2$ NPs concentration treatments. (E&F) Western blot analysis of hyaline (COL-II, SOX-9, and ACAN), (G&H) hypertrophic and catabolic (MMP-13, RUNX-2, and ADAMTS-5) phenotypes of chondrocyte after 24 h of Ta-NH$_2$ NPs treatment. (I) Morphology, Live/Dead, Krystal violet, Alcian blue, and COL-II/ACAN immunofluorescence staining of chondrocytes treated with Ta-NH$_2$ NPs. Data were represented as means ± s.d, from three independent replicates. **P<0.01.**
Figure 4

Ta-NH₂ NPs and CAT reduced oxidative stress and maintained the chondrogenic phenotype of H₂O₂-stimulated chondrocytes. Fluorescence staining of (A) ROS, iNOS, Live/Dead, ACAN and COL-II, (B) COL-I, ADAMTS-5, RUNX-2 and MMP-13 in H₂O₂ stimulated chondrocytes with or without Ta-NH₂ NPs and CAT pretreatments. (C) Western blot assay of protein expressions in above-described chondrocytes.
Quantitative analyses of fluorescence intensity and protein expressions. Data were represented as means ± s.d, from three independent replicates. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$

Figure 5

Metabolic kinetics of Ta-NH$_2$ NPs in knee joints and biodistribution in main organs of SD rats. In vivo fluorescence imaging of cy5.5-labeled Ta-NH$_2$ NPs and CAT in (A) intact, (B) dissected rat knee joints, and...
(C) femoral condyles from 1-28 days post single intra-articular injection. (D-F) Fluorescence quantifications of intact and dissected knee joints and femoral condyles post intra-articular injection. (H) Biodistribution of Ta-NH$_2$ NPs and corresponding (G) fluorescence quantification in major organs during 1-28 days post single intra-articular injection. Data were represented as means ± s.d. from three independent replicates.
**In vivo biocompatibility assessment of Ta-NH$_2$ NPs.** (A) Evaluation of in vivo toxicity of Ta-NH$_2$ NPs to major organs (heart, liver, spleen, and lung) at 1 d after knee joint injection. Serum levels of liver function indicators: aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) (B). 1 d and (D). 28 d after injection. Serum levels of kidney function indicators: blood urea nitrogen (BUN) and creatinine (CRE). (C). 1 d and (E). 28 d after injection. (F)–(M). Blood parameters in normal rat and rat knee joint injected with Ta-NH$_2$ NPs 1 d and 28 d after injection. In (B)–(M), data were represented as means ± s.d. from three independent replicates.

**Figure 7**

Ta-NH$_2$ NPs restored MIA-induced bone structure and alleviated the inflammation in synovium in rat model. (A) Schematic illustration of MIA-induced OA in SD rat. (B) Micro-CT images of the MIA pretreated knee joints after single articular injection of Ta-NH$_2$ NPs or CAT. (D) iNOS immunohistochemical and H&E staining of articular cartilage, subchondral bone, synovium and H&E staining of synovial tissue. The arrow points to the synovium and hyperplasia blood vessels. (E) Histopathological scoring of synovium at 4 and 8 weeks post treatments. Data were represented as means ± s.d. from three or five independent replicates (One-way ANOVA). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. 
Therapeutic efficiency of Ta-NH$_2$ NPs on MIA-induced OA. (A) H&E staining and (B) Safranin-O-Fast Green Staining of articular joints from each group. The box marked local magnification. OARSI score of (C) femur and (D) tibia at 4 and 8 weeks post treatments. Data were represented as means ± s.d. from five independent replicates (One-way ANOVA), *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$

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