Title: Nordihydroguaiaretic acid microparticles are effective in the treatment of osteoarthritis

Introduction

Osteoarthritis (OA) is a multifactorial degenerative joint disease that has remained a growing problem with rising incidence since the 20th century.[1, 2] Current treatments for OA include administering nonsteroidal anti-inflammatory drugs (NSAIDs) to alleviate pain and inflammation.[3, 4] However, no disease-modifying OA drug is available to date, as current treatments fail to prevent cartilage degradation and have several side effects.[5] One of the critical causative factors for OA is the accumulation of oxidative stress.[6, 7] Chondrocytes, the only resident cells of articular cartilage, produce reactive oxygen species (ROS) at basal levels *via* mitochondrial respiration and the NADPH oxidase system, vital for maintaining cartilage homeostasis.[8, 9]

Joint trauma or aging leads to the overproduction of reactive oxygen species (ROS), altering intracellular signalling pathways.[6, 9, 10] ROS mediates chondrocyte senescence and apoptosis, degradation of extracellular matrix, synovial inflammation, and erosion of the subchondral bone.[6, 11] Clinical data of OA patients aligns with these observations, showing elevated oxidative stress due to ROS and accumulation of lipid peroxidation products with decreased antioxidant enzymes.[12, 13] Excessive ROS can damage other cellular organelles resulting in cellular senescence or apoptosis.[14] Moreover, free radicals released from dysfunctional mitochondria can lead to telomere instability, pushing the chondrocytes into senescence.[15] Once the chondrocytes start becoming senescent, they secrete senescence-associated secretory phenotype (SASP) factors, which attract immune cells and lead to chronic inflammation and destruction of joint tissue.[16, 17]

Nordihydroguaiaretic acid (NDGA), a phenolic lignan extensively studied for its anti-cancer properties, emerges as a promising therapeutic to address these processes.[18-20] NDGA prevents lipoxygenase (LOX) activity by breaking the redox cycle of Fe^{3+} to Fe^{2+} conversion. This maintains the iron in the Fe^{2+} state to inactivate LOX and keeps it from generating ROS through its downstream products, thereby mitigating cellular damage.[21] NDGA is also a potent *in vitro* scavenger of several ROS species such as hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), hydroxyl molecules (OH^-), and nascent oxygen ([-O-]) and can prevent ROS-mediated cellular damage.[22] NDGA modulates the nuclear factor erythroid 2-related factor 2 (Nrf2) anti-oxidant pathway, helps reduce the intracellular oxidative stress damage to delay senescence and helps prevent age-related diseases.[23, 24]

Autophagy, another critical aspect of OA, represents a cellular homeostasis mechanism that ensures cellular stress survival by balancing the catabolic and anabolic pathways.[25-29] In the context of OA, autophagy is an emerging area of therapeutic research that involves the upregulation of autophagy by mTOR inhibition, thereby recycling ROS-damaged organelles and preventing cellular damage. [27, 30-34] Autophagy upregulation *via* mTOR inhibition prevents further cellular damage by recycling ROS-damaged organelles.[35]

A key study by the National Institute on Aging's Interventions Testing Program identified NDGA as a treatment to prolong the life span of mice by acting as an autophagy modulator and preventing cellular aging.[23, 36] EP300 acetyltransferase is one of the essential enzymes causing raptor acetylation leading to mTORC1 activation and autophagy inhibition.[37-39]

NDGA induces autophagy *via* EP300-mediated mTORC1 inhibition to recycle the ROS-damaged and dysfunctional organelles, avoiding their accumulation and can prevent senescence onset in cells.[23, 40] NDGA also partially inhibited MMP-13 production in chondrocytes caused by fibronectin fragments and reduced nuclear factor-B and MAP kinase phosphorylation, thereby preventing the development of the self-perpetuating inflammation-resolution cycle that contributes to the development of OA.[41] NDGA also has been shown to prevent ionizing radiation-mediated oxidative stress-induced senescence in articular chondrocytes *in vitro*.[42] Since OA is a disease with multiple contributing factors such as increased oxidative stress, imbalanced autophagy, and chondrosenescence, NDGA has the potential to act as a DMOAD candidate.[21] However, NDGA has not been used to treat OA in any animal model, perhaps due to its low molecular weight, which poses the challenge of low residence time in the joint.

Novel drugs and biologics such as tanezumab and anakinra that were successful in OA preclinical studies, have not been successful in human trials; a major reason is attributed to systemic toxicity, rapid clearance, and sub-therapeutic concentrations in the joints.[43-47] Administering frequent intra-articular injections can help sustain therapeutic concentrations but does not offer a patient-compliant therapy. Microparticle-mediated drug delivery using sustained-release formulations is a promising approach to overcome this challenge. The clinical success of sustained-release formulations in several disease conditions is supported by studies involving drugs such as doxycycline (NCT02487186), triamcinolone acetonide (NCT04094298), and Zilretta®, risperidone (Risperdal Consta®), and naltrexone (Vivitrol®). Hence, given NDGA's potential benefits, we hypothesized that biomaterial microparticle-based intra-articular delivery of NDGA would result in sustained delivery and improved therapeutic efficacy for the treatment of OA. Although few reports have explored sustained-release formulations of NDGA [48, 49] none have investigated its application for OA treatment.

Herein, we report that the NDGA retained bioactivity after encapsulation in microparticle formulation, prevented senescence, and induced autophagy in human primary chondrocytes obtained from OA patients. Microparticle formulations of NDGA resulted in sustained release of NDGA for several weeks. Both preventive and curative regimens with NDGA-microparticle formulations effectively ameliorated post-traumatic OA in a mouse model by inducing autophagy, inhibiting LOX production, and preventing senescence in articular chondrocytes. These results suggest a promising avenue for treating OA using NDGA microparticle formulations.

Objectives:

1. Engineer PLGA-based small molecule carriers to ensure a controlled and sustained release of the Nordihydroguaiaretic acid (NDGA) over an extended period
2. Assess the impact of NDGA in free and microparticle formulation on senescence and sulphated glycosaminoglycan (sGAG) production *in vitro*
3. Evaluate the effectiveness of NDGA carriers in the mouse model of osteoarthritis and analyse disease progression by characterizing cartilage damage and symptomatic relief

Materials and Methods: A brief description of the methods used in the study is presented here.

Synthesis of PLGA microparticles: PLGA microparticles were synthesized using a single emulsion technique with PLGA polymers of different molecular weights (10-15 kDa, 85-100

kDa, and 190-240 kDa). The release studies were performed in 1x PBS. For *in vitro* studies, NDGA (3 mg) encapsulated in 10 -15 kDa PLGA was used and for *in vivo* studies, NDGA (5 mg) encapsulated in 85-100 kDa PLGA was used for therapeutic delivery.

Isolation of human articular chondrocytes from human knee joint surfaces: Knee joints excised during total knee arthroplasty surgery were immediately brought to the IISc tissue culture facility and processed for articular chondrocyte isolation. The isolated cells were cultured and used within the first 3 passages for most of the experiments.

WST assay: Human Chondrocyte cells (C28/I2) plated in a 96-well plate were treated with increasing concentrations of NDGA for 24 h or 48 h. Post incubation timepoint, WST reagent was added, and absorbance readings were measured at 470 nm.

Senescence induction assay: Primary human articular chondrocytes (HACs) were treated for 48h with oxidative stress agent- hydrogen peroxide (H_2O_2) (200 μM) to induce senescence. Colorimetric SA-β Gal activity was used to stain senescent cells as previously described [50]. Bright-field images were taken from each treatment group and a macros algorithm was used to count the total number of senescent cells in ImageJ.

sGAG production (Micromass culture): The HACs were seeded as a 15 μL suspension in growth media in a 24-well plate, allowed to adhere, and treated with H_2O_2 (100 μM) and co-treated with free NDGA (3 μM) or NDGA-MP (final concentration 3 μM) for 48h. Post incubation, Alcian Blue staining was used to quantify the sGAG produced by the different treatment groups.

The residence time of PLGA MPs in mouse knee joint: PLGA microparticles (85 kDa - 100 kDa with Cy7 dye) were injected in mice's left knee joints by intra-articular injections on Day 0 and the contralateral legs received free dye injection. Fluorescence imaging was done using IVIS® Spectrum starting at Day 0 until Day 35 to evaluate the residence time of PLGA MPs in mice knee joints.

PTOA Mice model: The widely used DMM surgery to induce PTOA in mice was used in this study. The DMM-operated mice were treated with NDGA as a free drug or in MP formulation administered as an intra-articular injection at predetermined time points to maintain therapeutic levels.

Histopathology: The fixed mice knee joints were embedded in paraffin and five-micron sections were stained with Safranin O staining or immunohistochemical staining for several antibodies such as MMP-13, ADAMTS-5, LC3B, 15-LOX, and p19ARF antibodies.

MicroCT experiments: Mice knee joints were mounted in Bruker SKYSCAN 1272 and scanned at preset voltage/current as 10 μm voxels to obtain tomographs that were reconstructed using Scanco Medical evaluation software and further evaluated for several morphometric parameters.

Assessment of Pain: Mechanical sensitivity of knee joints post DMM surgery was evaluated before and after treatments to assess the functional evaluation of the knee joints using Von Frey Anesthesiometer.

Statistical Analysis: All statistical analyses were performed with GraphPad Prism software (8.0.2). One-way ANOVA was performed when comparing three or more groups. Statistical significance and sample size (*n*) for each analysis are listed in figure legends.

Results

PLGA microparticles as tunable NDGA delivery platform: A sustained release formulation of NDGA can result in a lower frequency of drug administration for the long-term treatment of OA. PLGA has been widely used as sustained drug delivery formulation due to its excellent biocompatibility and ease of tunability to encapsulate and sustainably deliver a wide variety of drugs.[51, 52] We hypothesised that, since NDGA is a hydrophobic drug with a low water solubility, encapsulating it in the PLGA platform using the single emulsion technique can create a slow-release platform *via* PLGA degradation to deliver higher therapeutic cargo to the target site. This method of NDGA encapsulation in PLGA matrix has not been evaluated before.

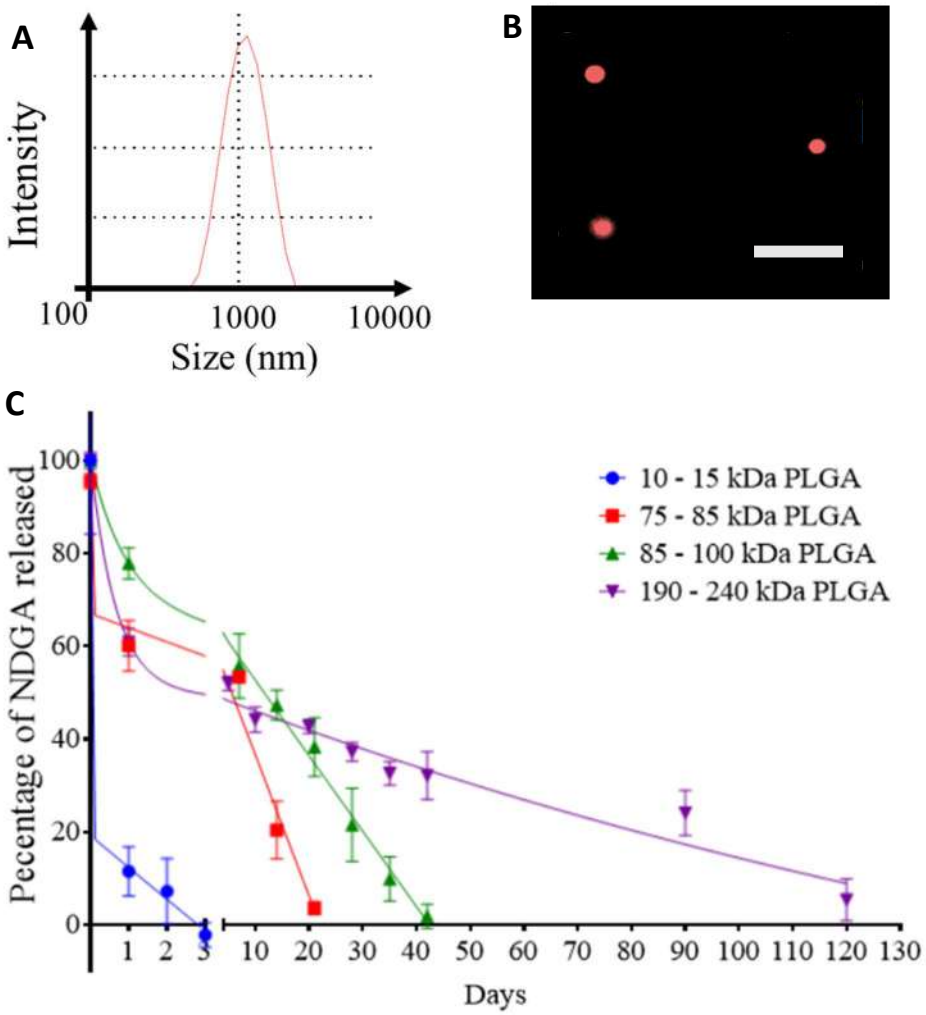

Fig. 1. Characterization of PLGA particles as NDGA carriers (A) Size distribution of 10 - 15 kDa PLGA particles measured by Dynamic Light Scattering (DLS). **(B)** Fluorescence microscopy image of Cy3 loaded PLGA particles (molecular weight 10 - 15 kDa), Scale bar, 3 µm. **(C)** *In vitro* release profiles of NDGA from microparticles synthesized from different molecular weight PLGA polymers (n = 3 per group at each time point). Data in the graph were fitted with non-linear regression (least square method) two-phase exponential decay curves.

Table 1: PLGA microparticles of different molecular weights and PLA: PGA ratios, their respective sizes, and their NDGA encapsulation efficiency.

Molecular weight of PLGA (Ratio of PLA: PGA)	**Size - DLS [nm]**	**NDGA encapsulation efficiency [%]**
10 kDa - 15 kDa (50:50)	**939.8 ± 335.3**	**8.98 ± 1.316**
85 kDa - 100 kDa (50:50)	**1139 ± 407.6**	**8.84 ± 0.565**
190 kDa –240 kDa (85:15)	**1118 ± 335.3**	**6.22 ± 0.745**

Our previous studies have shown that the microparticles (MPs) with 1 µm diameter exhibit prolonged retention inside mice and rodent knee joints.[33, 34, 53] Hence, we prepared PLGA particles (molecular weight: 10 – 15 kDa; encapsulation) with an average hydrodynamic diameter of 939.8 ± 335.3 nm (**Fig. 1A** and **Table 1**). The fluorescence image of Cy3 loaded PLGA MP is shown in **Fig. 1B**.

Next, we determined whether the release rate of NDGA could be varied with PLGA microparticles of different molecular weights (Mw). The size and NDGA encapsulation efficiency in various PLGA MPs formulations are listed in **Table 1**. From the NDGA release profiles, it was evident that within 48 h, 10 - 15 kDa PLGA MPs released all the drug, while the 85 - 100 kDa PLGA MPs of Mw released the drug for 45 days. With variation in the PLA: PGA ratio of the PLGA from 50:50 to 85:15 and using higher molecular weight PLGA (190 - 240 kDa), we could sustain the NDGA release up to 120 days (**Fig. 1C**). Thus, adjusting the molecular weights and the PLA: PGA ratio allowed NDGA release in a controlled and sustained manner, providing a robust and versatile platform. For 48 h *in vitro* experiments, the shorter releasing particles (10 – 15 kDa) were used.

NDGA cytocompatibility with C28/I2 cells: Before beginning to assess the NDGA and NDGA-MP therapeutic effect on chondrocytes, we sought to test the chondrocytes' metabolic activity in the presence of various concentrations of NDGA to establish a safe range for our *in vitro* experiments. We treated the human chondrocytes cell line (C28/I2) to different concentrations of NDGA for 24 and 48 h (**Fig. 2A and B**). Cellular metabolic activity did not reduce for concentrations up to 30 µM at the end of 48 h. Therefore, for all future experiments, we decided to work with the range of 200 nM to 30 µM.

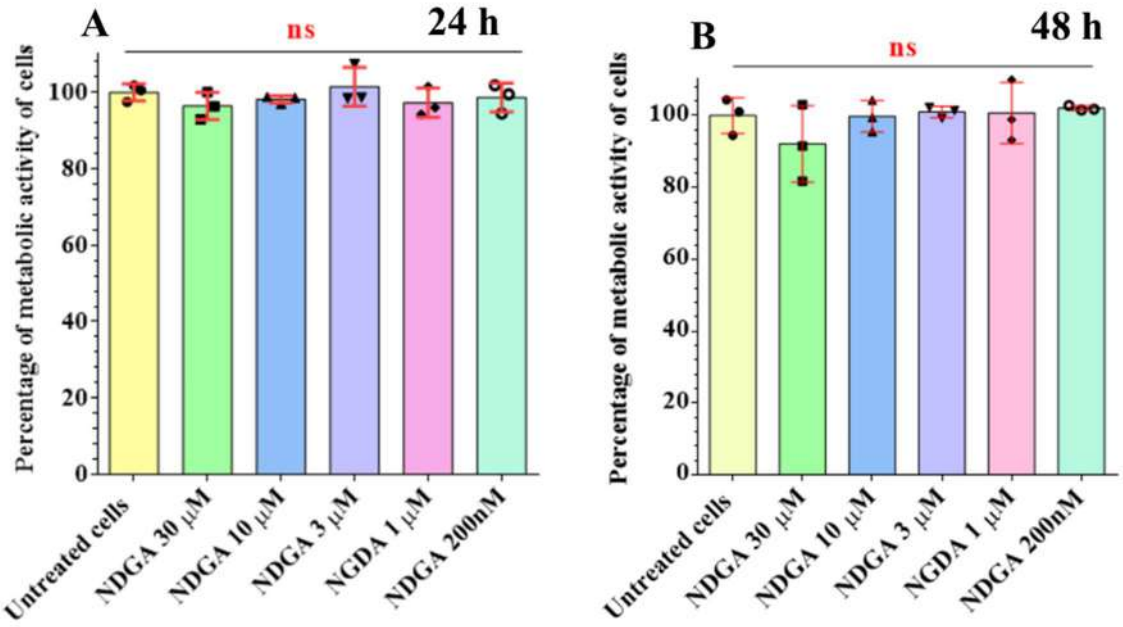

Fig. 2. Cytocompatibility of NDGA. Metabolic activity of chondrocytes treated with varying concentrations of NDGA for **(A)** 24 h and **(B)** 48 h.

NDGA-MP prevented senescence in HACs: Previous studies conducted in our lab had showed the activity of NDGA in upregulating autophagy and reducing lipoxygenase activity. We hypothesized that NDGA, a potent ROS quencher, can prevent oxidative stress build-up in the chondrocytes thereby preventing senescence. We treated the HACs under oxidative stress conditions by externally adding H_2O_2 (200 μM) to test this hypothesis. The concentrations of stress agents such as H_2O_2 and BrdU were previously optimised.[33] The experimental results indicated that NDGA could prevent senescence. To evaluate the senescence prevention capability of NDGA as a microparticle formulation, we co-treated HACs exposed to H_2O_2 (200 μM) with NDGA-MP (NDGA equivalent to 3 μM). The H_2O_2 (200 μM) treatment increased the percentage of senescent cells to 35.67 ± 5.44 %, while the free NDGA and NDGA-MP co-treated groups decreased the percentage of senescent cells to 6.88 ± 2.91 % and 6.66 ± 0.82 % respectively compared to untreated cells, which showed 6.55 ± 2.08 % senescent cells (**Fig. 3A to E).** Thus, NDGA as free drug or in microparticle formulation prevented senescence under stress conditions.

NDGA-MP treatment sustained sGAG production in stressed micromass cultures: We next wanted to evaluate whether chondrocyte function can be restored under oxidative stress. To evaluate this, we performed functional assays to evaluate the sulfated glycosaminoglycans (sGAG) production in the oxidatively stressed chondrocytes. When seeded at very high densities in the presence of the growth factor (TGF-β), chondrocytes form 3D micromasses with high deposition of extracellular matrix components including sGAG[54]. There was a 2.6-fold decrease in the sGAG production by the micro masses with H_2O_2 treatment compared to the vehicle-treated groups (**Fig. 3F**). The free NDGA or NDGA-MP treatment both sustained the sGAG production at levels comparable with untreated groups. In summary, these results suggest that NDGA and NDGA-MP sustained sGAG production in stressed chondrocytes for long durations under oxidative and genotoxic stress.

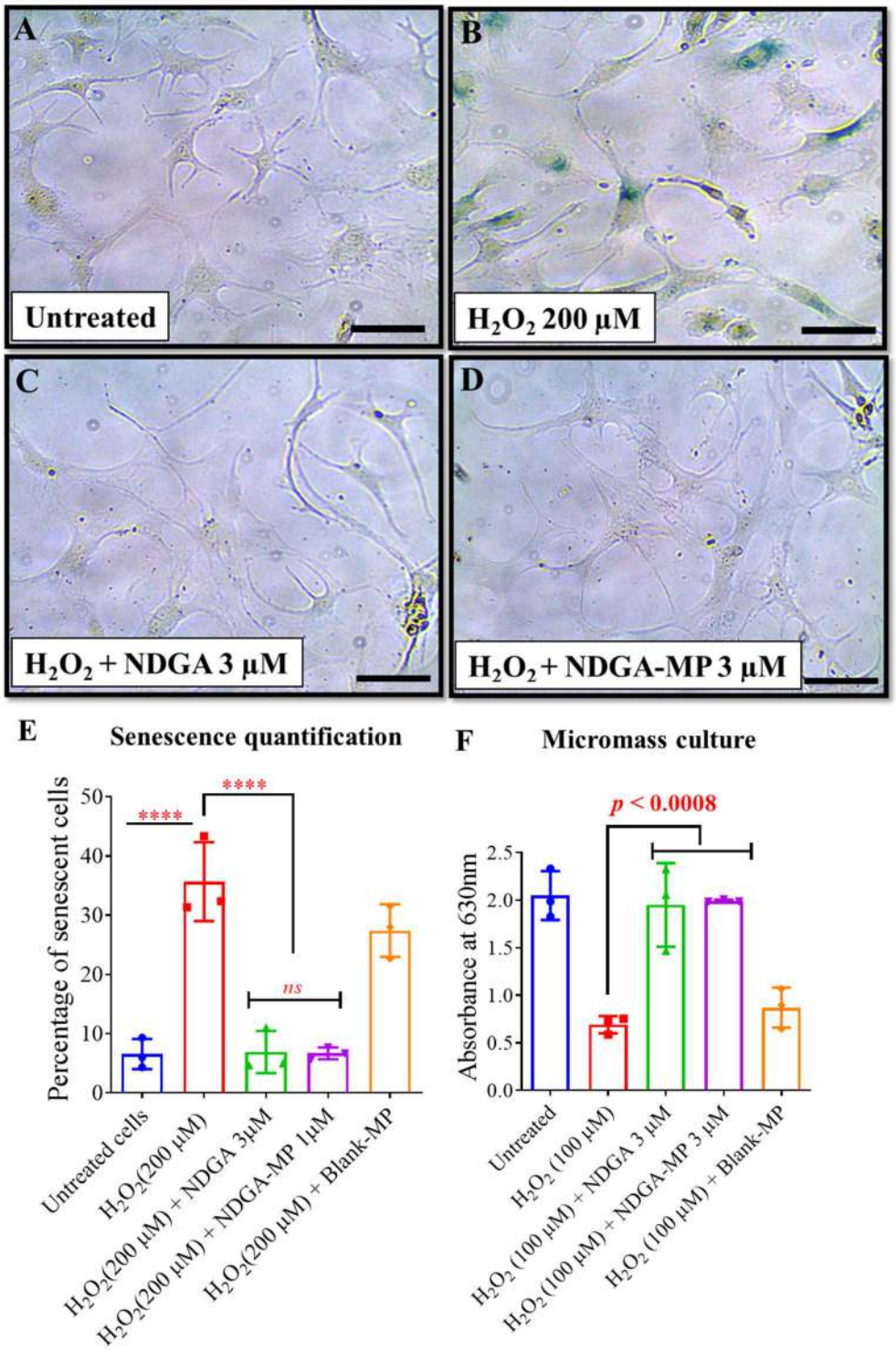

Fig. 3. NDGA-MP prevented senescence in primary HACs and sustained sGAG production in micromass cultures exposed to oxidative stress. SA-β Gal-stained images of primary HACs exposed to **(A)** no treatment, **(B)** oxidative (H_2O_2) stress, **(C)** oxidative (H_2O_2) stress with free NDGA (3 µM), and **(D)** oxidative (H_2O_2) stress with NDGA-MP (3 µM). **(E)** Percent of senescent HACs post oxidative (H_2O_2) stress condition for 48h with various treatment conditions (n = 3 per group). **(F)** Absorbance of Alcian blue stain from stressed micromass cultures (n = 3 per group). Images and graphs were representatives of data collected from three OA patients. Data in graphs represent the mean ±, s.d. and *p* values were determined

by one-way analysis of variance (ANOVA) and Tukey's post hoc tests. *p*-value < 0.05 was considered significant. HACs – Human Articular Chondrocytes, Blank-MP - Blank Microparticles, NDGA-MP – NDGA loaded Microparticles. ****p<0.0001, *ns* – non significant. Scale bar, 20 µm.

PLGA MPs increased residence time of small molecule in mice knee joints: We wanted to determine the retention time of the drug in mice knee joints when delivered using PLGA microparticles before proceeding with the in vivo assessment of the formulation. Since the 85 – 100 kDa PLGA MPs displayed a sustained release pattern for more than a month (**Fig. 1C**), we chose this Mw PLGA formulation for all subsequent mice experiments. Since the longer releasing particles (190-240 kDa) released NDGA for about 120 days, we did not employ it in this study. To determine the residence time of PLGA MPs in the knee joint, fluorescent Cy7 amine dye encapsulated in PLGA MPs was administered intra-articularly, and the fluorescence signal was monitored using an *in vivo* imaging system – Perkin Elmer IVIS® Spectrum. The contralateral legs of mice were injected with an equal amount of Cy7 free dye**.** On day 3, the free dye injected joints only had 10% of the initial signal, whereas the Cy7 MPs injected group exhibited fluorescent signal even on day 35 (**Fig. 4A-B**). Collectively, the results demonstrate the feasibility of using microparticles for prolonged delivery of molecules into the intra-articular space. We had previously reported similar retention times for 1 µm PLGA particles (75-85 kDa).[33, 34]

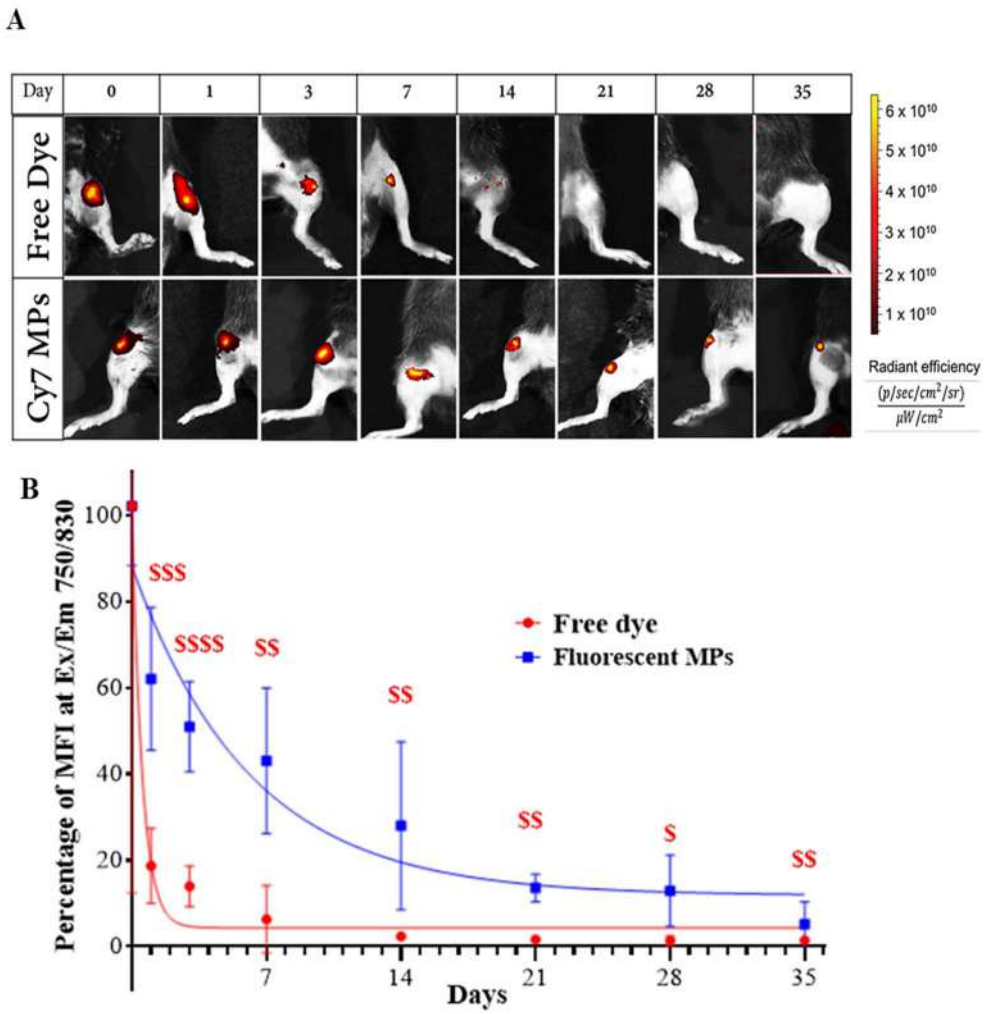

Fig. 4. PLGA MPs exhibit prolonged residence time in mice knee joints. **(A)** *In vivo* images of mice, knee joints imaged at day 0, 1, 3, 7, 14, 21, 28, and 35 after intra-articular injection of free Cy7 dye (top panel) and Cy7-labeled 85 – 100 kDa PLGA MPs (bottom panel). **(B)** Percentage of fluorescent intensity remaining from injected formulations for days (n = 5 mice per group). Data in the graph were fitted with non-linear regression (least square method) one-phase exponential decay curve. Data in the graph represent the mean ± s.d., and *p* values were determined by unpaired t-test or Mann-Whitney test. *P*-value < 0.05 was considered significant. **$** - 0.0159, **$$** ≤ 0.0079, **$$$** - 0-0006, **$$$$** ≤ 0.0001.

The curative regimen of NDGA-MP reduces the severity of OA in a murine post-traumatic model of OA: The animal experiments were designed as a 2-month model of OA based on previous studies and we administered free NDGA (4 µg per joint at each time point), NDGA-MP (1 µg per joint at each time point), and Blank-MPs (MPs equivalent to the injected particle dose of NDGA-MP) intra-articularly as 2 injections, formulation (**Fig. 5A**). The residence time of free drug in mice joints is very less, therefore, we used 4 times higher dose of free drug as compared to microparticle formulation for all *in vivo* studies.

Orthopedic clinics receive patients who arrive with severe symptoms due to progressive damage of the existing knee joint.[55] Hence, to hold more relevance for treating the patients at the clinics, we wanted to evaluate whether our NDGA formulations would be effective after the disease has progressed to early OA.

Studies have shown that OA hallmarks such as proteoglycan loss, chondrocyte hypertrophy, and cartilaginous osteophyte formation are detectable as early as two weeks post-surgery in DMM mice models.[56] Therefore, we administered the DMM-operated mice groups with NDGA-MP formulation three weeks post-DMM surgery to assess if the formulation can cure the early OA damage. The frequency of injections was kept to two injections spaced 21 days apart (**Fig. 5A**). We monitored the drug toxicity-related weight loss in mice. All the animals showed a steady weight gain throughout the study period (**Fig. 8A** and **B**).

DMM operated groups with no treatment, Blank-MPs treatment, and free NDGA treatment showed cartilage surface irregularities with significant cartilage thinning. There was diminished safranin O staining of proteoglycans in the cartilage along with hypertrophic chondrocytes exhibiting focal perichondral staining or empty lacunae, indicating chondrocyte death (**Fig. 5B**). The OARSI scores in mice receiving two-dose injections of NDGA-MP were three times lower than those treated with free NDGA (p <0.0001) and DMM only (p <0.0001) (**Fig. 5C**). We observed that administration of NDGA-MP effectively alleviated the allodynia than free NDGA and untreated groups. Untreated and free drug administered group had a low pain threshold (<2 g) whereas NDGA-MP injected animals showed similar pain thresholds to surgical and healthier controls (**Fig. 5D**).

The immunohistochemical studies provide additional confirmation of our *in vitro* findings. The cells positive for 15-LOX **(Fig. 5E and F)** and p19ARF **(Fig. 5I and J)** were 3-fold lower in the mice joints treated with 1 µg NDGA-MP than free NDGA (p = 0.0001) or DMM only (p = 0.0001) groups There were fewer senescent cells in the DMM and Blank-MP-treated groups but were not statistically different from the free NDGA-treated group. NDGA-MP treatment also induced an upregulation of basal autophagy in the chondrocytes on the surface of the articular cartilage in mice treated with NDGA-MP (1 µg), evidenced by an increased number

of LC3B-positive cells. In contrast, the free NDGA and Blank-MP treated groups exhibited fewer LC3B positive cells indicative of low basal autophagy (**Fig. 5G** and **H**).

Single intervention study: Finally, we wanted to evaluate if we can further reduce the dosing frequency of our particles to achieve therapeutic benefits. Hence, we designed a single injection dosing regimen (**Fig. 6A**). Under the single intervention regimen, mice treated with NDGA-MP exhibited OARSI scores that were 2-fold lower than those treated with free NDGA ($p < 0.0001$) and DMM only ($p = 0.027$) (**Fig. 6B and C**). Thus, NDGA-MP at a dose of 1 μg per joint effectively reduced improved overall cartilage health. We also checked for pain threshold which did not improve with NDGA-MP or free NDGA injections compared to DMM group (**Fig. 6**). μCT analysis was performed to determine changes in subchondral bone and osteophytes (**Fig. 7**). The analysis revealed osteophyte formation across different groups (**Fig. 7A-D**), and the quantification of osteophyte volume showed no significant differences between the DMM and microparticle-treated groups (**Fig. 7F**), indicating that the formulation did not prevent osteophyte formation. Similarly, measurements of subchondral bone volume showed no significant differences across all groups (**Fig. 7E**).

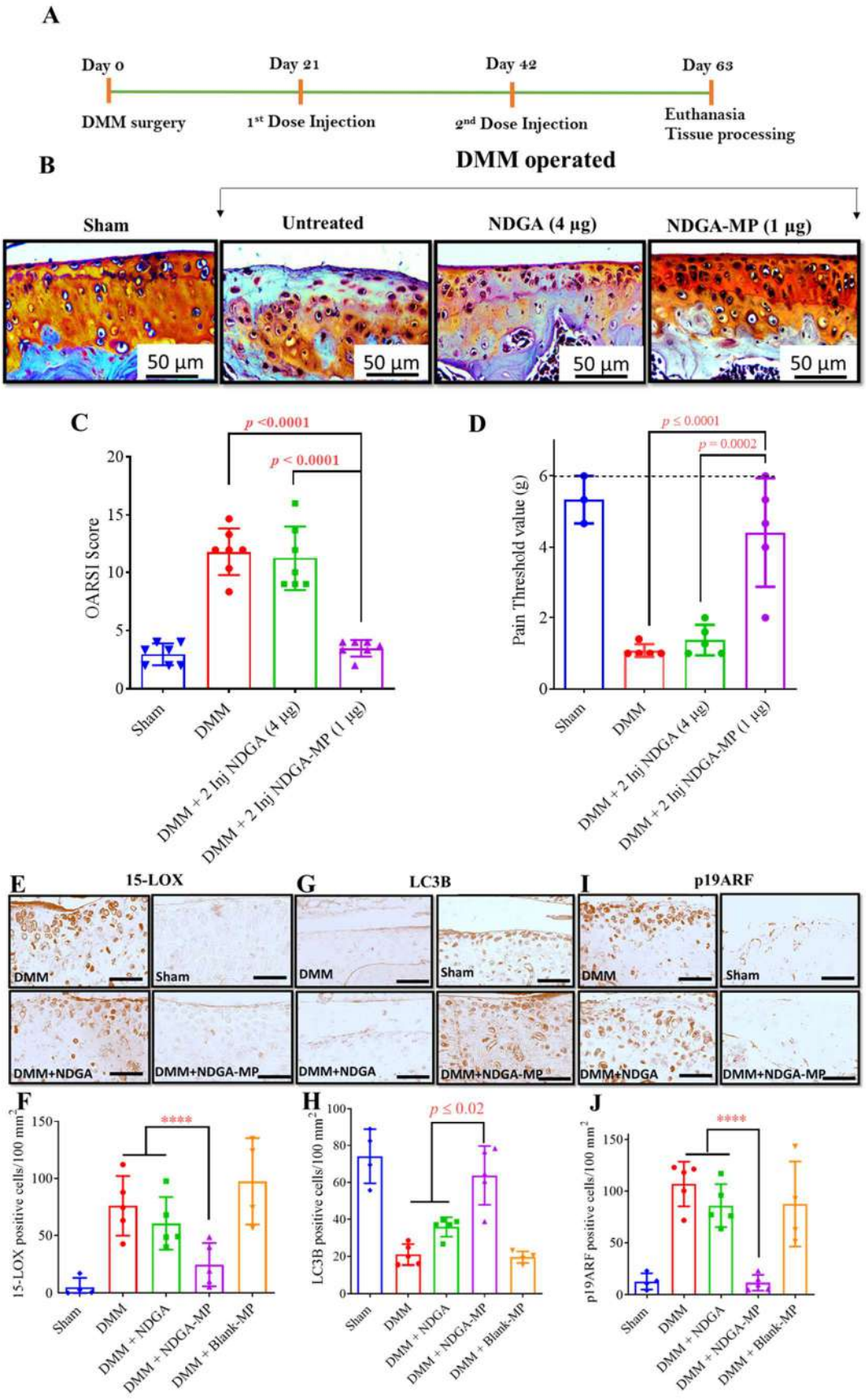

Fig. 5. NDGA-MP as a curative regimen reduced the severity of OA in the murine post-traumatic model of OA (A) Graphic illustration of treatment timeline. **(B)** Characteristic

Safranin-O and Fast Green–stained images of medial tibial plateau (MTP) of mice knee joints collected from the different treatment groups. **(C)** OARSI scores indicate the extent of injury in mice after receiving different treatments. (**D**) Pain threshold of different groups of mice treated with a curative two dosing regimen at 9 weeks post DMM surgery and dotted line representing pain threshold of unoperated mice. **(E)** Characteristic images of 15-LOX IHC staining **(F)** Quantification of 15-LOX positive cells per 100 mm^2. **(G)** Characteristic images of LC3B IHC staining. **(H)** Quantification of LC3B positive cells per 100 mm^2 **(I)** Characteristic images of p19ARF IHC staining. **(J)** Quantification of p19ARF positive cells per 100 mm^2. The data in the graphs represent the mean ±, s.d., and *p* values were determined using one-way analysis of variance (ANOVA) or Kruskal Wallis test and Tukey's post hoc analysis. DMM (n = 7), sham (n = 7), free NDGA (n = 7), NDGA-MP (n = 7), Blank-MPs (n = 7). *ns* - non significant; Scale bar, 50µm.

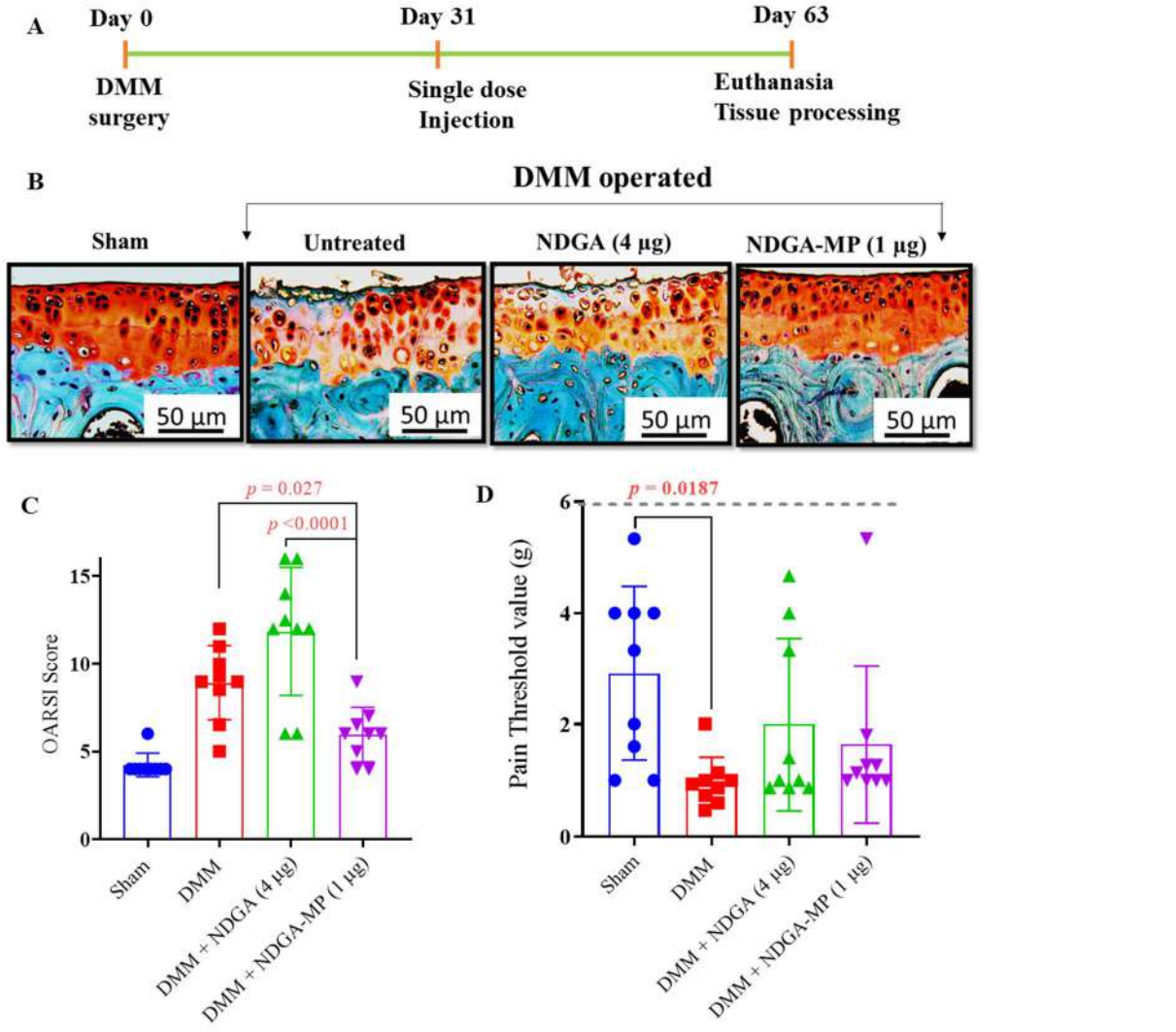

Fig. 6. NDGA-MP as a curative single-dose regimen reduced the severity of OA in the murine post-traumatic model of OA. (A) Graphic illustration of treatment timeline. **(B)** Characteristic Safranin-O and Fast Green–stained images of the medial tibial plateau (MTP) of mice knee joints collected from the different treatment groups. **(C)** OARSI scores indicate the extent of injury in mice after receiving different treatments. **(D)** Pain threshold of different

groups of mice treated with a curative single dosing regimen at 9 weeks post DMM surgery. The data in the graphs represent the mean ±, s.d., and p values were determined using one-way analysis of variance (ANOVA) or Kruskal Wallis test and Tukey's post hoc analysis. DMM (n = 9), sham (n = 9), free NDGA (n = 9), NDGA-MP (n = 9), Blank-MP (n = 9). ns - non significant; Scale bar, 50µm.

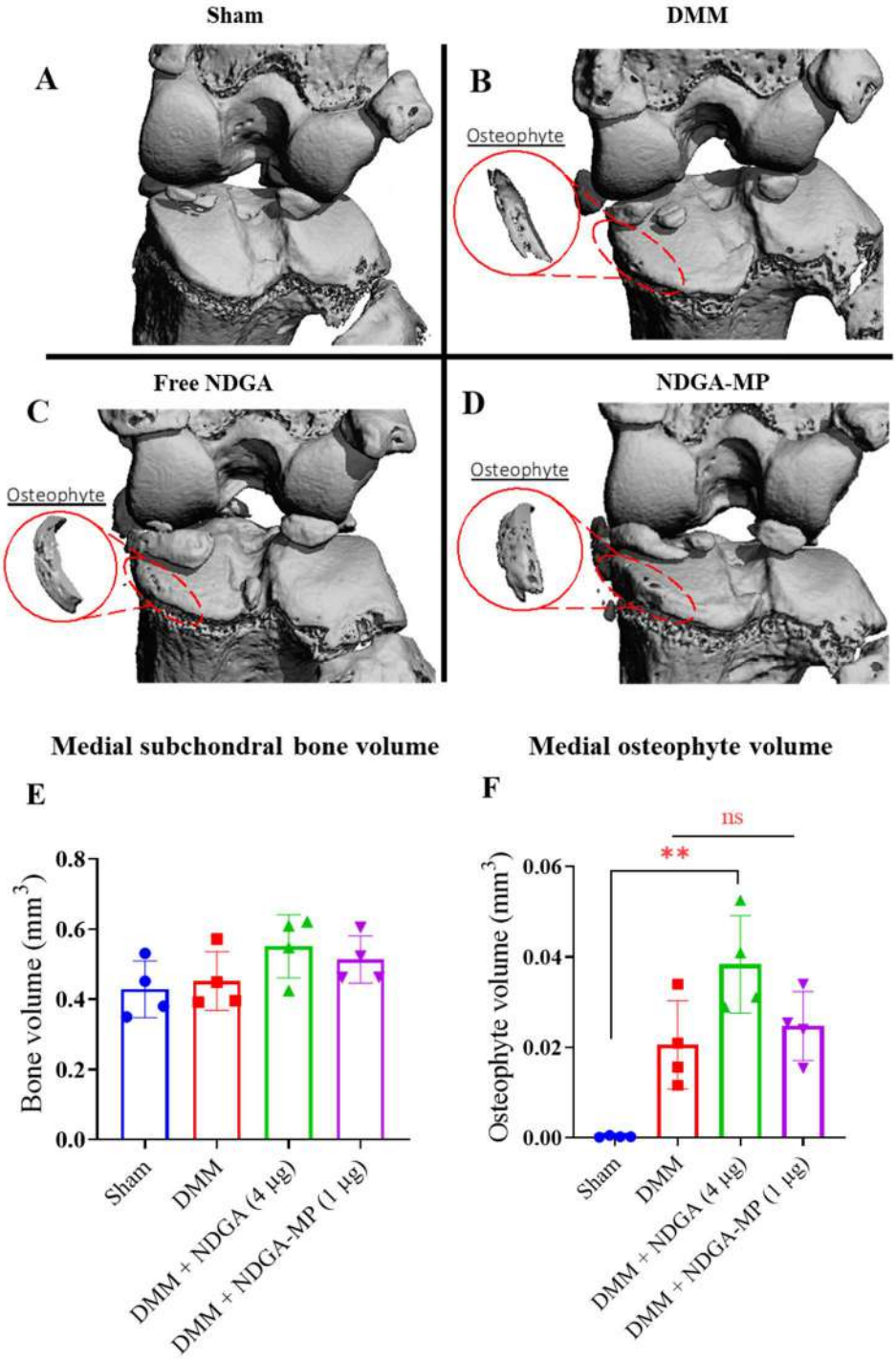

Fig. 7. µCT analysis of joints obtained after single dose regimen in the murine post-traumatic model of OA. Representative 3D posterior whole joint reconstructions from µCT analyses with medial tibial plateau marginal tissues (osteophytes) highlighted in red collected

from **(A)** sham, **(B)** DMM, **(C)** NDGA and **(D)** NDGA-MP. **(E)** Medial subchondral bone volume in mice treated with a curative single dosing regimen showed no statistical difference among any groups. **(F)** Medial osteophyte bone volume in mice treated with a curative single dosing regimen did not decrease the osteophyte volume compared to the DMM group and were all significantly higher compared to the sham group in statistical analysis. The data in the graphs represent the mean ±, s.d., and *p* values were determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis or Kruskal-Wallis test followed by Dunn's post hoc analysis. n = 4 for all groups.

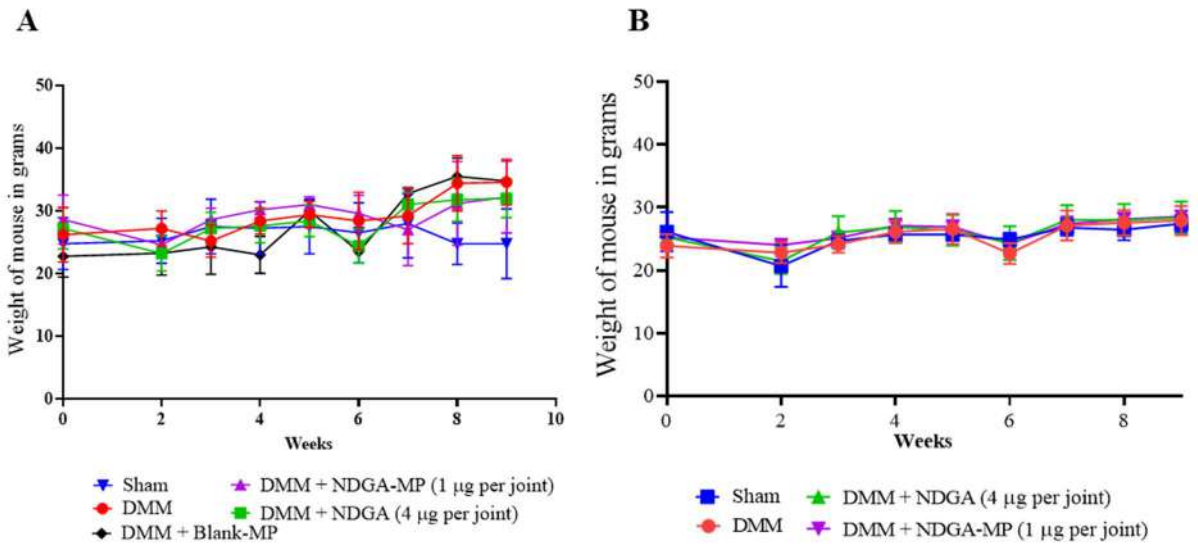

Fig. 8. Mice treated with NDGA as a Free Drug and as NDGA-MP do not cause toxicity-related loss of body weight. (A) Plot showing the weight of mice for the time after receiving different treatments under a curative two-dose regimen. **(B)** Plot showing the weight of mice for the time after receiving different treatments under a curative single-dose regimen.

Discussion

Given the progressive nature of OA and the lifelong morbidity, treating joints post-trauma with disease-modifying drugs can prevent severe OA. New emerging agents targeting specific etiologies of OA, such as the Interleukin-1 receptor antagonist (IL-1 RA), MMP–inhibitor PG-116800 (NCT00041756), anti-IL-1α/β antibody – Lutikizumab (NCT02087904), and novel DMOAD drugs such as UBX0001 (NCT04129944), Enbrel (NCT02722772), kartogenin and tanezumab, did not work efficiently in clinical trials [45, 57, 58], possibly due to rapid joint clearance.

Intra-articular drug injection *via* a sustained release carrier could represent a more practical and safer alternative. Our study identified a novel DMOAD, NDGA, which has not been previously used in animal models for OA research. NDGA induces autophagy that recycles the damaging ROS molecules and damaged organelles to restore cellular health.[23, 59, 60] Despite its great curative potential, NDGA can cause hepatotoxicity and nephrotoxicity if administered at high

doses. [59, 61] Hence, a slow and a carrier mediated sustained local delivery will help prevent these toxic effects. Using this novel DMOAD, we have successfully engineered a sustained release microparticle formulation that maintained therapeutic concentrations in the knee joint. Our study treated the PTOA mouse model by administering IA injection twice and eventually showing that a single injection over two month OA model is sufficient to provide curative benefit. Such dosing frequencies have been clinically acceptable such as Hyalgan® administration (NCT00669032, 3 cycles of weekly intra-articular injections for 5 weeks) or the current clinical trials for bioactive agents such as DA-5202 (NCT02554240, weekly intra-articular injection for 3 weeks).

The excellent biocompatibility and biodegradability of PLGA make them a suitable carrier for NDGA delivery. In addition, PLGA encapsulated formulations, like Zilretta®, Lupron®, and Zoladex®, have been approved by the US Food and Drug Administration (FDA) as a safe formulation with no adverse effects for OA and other clinical therapies. Our previous studies have demonstrated that particles in the 900 nm – 1 μm size range are appropriate for retention in mice or rodent knee joints *via* intra-articular administration.[33, 34, 53] To evaluate the retention time of these particles *in vivo*, we used microparticles synthesized from 85 - 100 kDa molecular weight PLGA. The MPs exhibited extended residence time (~35 days) in murine knee joints (**Fig. 4**). Further investigation into PLGA particles made from even higher molecular weight polymer may exhibit longer residence time in the knee joints for effective translation into larger animal models and eventually humans.

The various etiologies of OA are trauma due to sudden impact, and aging that can cause a surge in ROS production and diminished autophagy.[27, 62-66] Previous studies have demonstrated that 15-LOX was upregulated in OA under trauma and hypoxic conditions, and its prime metabolite 15(S)-HETE is implicated in mediating the surge in ROS production.[67] Autophagy plays an essential role by actively recycling the ROS molecules and the dysfunctional mitochondria and stressed endoplasmic reticulum.[25-27, 35] With trauma to the knee joints, the chondrocytes undergo autophagic imbalance leading to cellular senescence and impairment of the cartilage repair process.[27, 68] NDGA is a cell-permeable anti-oxidant, autophagy up regulator, and selective LOX inhibitor. It prevents the accumulation of damaging ROS species and helps maintain cellular homeostasis.[21, 69, 70]. In previous experiments conducted in lab, it was already established that NDGA was able to reduce lipoxygenase activity and increase autophagy in HACs.

Chondrosenescence, another important contributing factor of OA disease due to ROS accumulation, dysfunctional organelles, and poorly upregulated autophagy.[71] For the first time, we provide evidence in primary HACs that NDGA-MP can prevent oxidative stress-induced senescence (**Fig. 3A to E**) and sustain sGAG production (**Fig. 3F**). Similarly, *in vivo* studies showed a reduced number of p19ARF in the NDGA-MP treated group, indicating fewer senescent cells (**Fig. 5I and J**). Thus, NDGA-MP administration into mouse knee joints effectively attenuated surgery-induced cartilage damage (**Fig. 5B-C)** and reduced clinical manifestation of pain **(Fig. 5D)**. We also attempted a single dose regimen administered a month after the surgery and observed continued protection against cartilage damage (**Fig. B-C**), however delayed treatment with NDGA-MPs failed to significantly improve pain scores or reduce osteophyte volume (**Fig. 6D and 7**). These data suggest that a single administration of the NDGA-MP formulation may be insufficient to completely rescue bone-related degeneration in more severe cases of established OA. Further investigation is required in larger animal

models where the disease development is slower compared to mice. Several potent and safer analogues of NDGA are also described and can also be tested for the treatment of OA[48, 59, 72]. Additional studies are needed to evaluate the dosing regimens and effectiveness in higher animal models for progression into the clinics.

In summary, we report NDGA in a sustained release formulation has the potential to be a DMOAD to ameliorate post-traumatic OA. The relevance of our results to human disease was corroborated using chondrocytes isolated from osteoarthritic patients. This study opens up avenues of using autophagy inducers, ROS quenchers and senescence prevention for the treatment of OA.

Impact of the research in the advancement of knowledge or benefit to mankind

Our study represents a significant advancement in OA therapy by introducing NDGA as a novel disease-modifying osteoarthritis drug (DMOAD) candidate. Unlike the existing treatments that primarily manage symptoms, NDGA targets multiple mechanisms, including the upregulation of autophagy, scavenging of reactive oxygen species (ROS), and preventing senescence, thereby offering a new approach to target the disease. Our research provides critical insights into OA pathophysiology, particularly the link between autophagy, senescence, and inflammation. Also, it highlights the importance of maintaining cellular homeostasis by combating cellular stress. NDGA-MP protects the articular chondrocytes from damage and sustains sGAG production, essential for OA prevention. The single injection and two injection administration studies demonstrated the potential of NDGA-MP in protecting the cartilage from degradation during OA progression and alleviating OA symptoms, thereby reducing the need for multiple injections or invasive surgeries currently offered in clinics.

Further, it also emphasizes the need for early intervention in post-traumatic osteoarthritis (PTOA) by showing that administration of 2-dose injections of NDGA-MP can effectively reduce cartilage damage and alleviate OA symptoms. In addition to its impact on OA treatment, our research shows the clinical translational potential of small molecule drugs using microcarriers of PLGA, an FDA-approved biocompatible polymer. Overall, our study offers potential translation of the small molecule NDGA in microparticle formulation into clinics to offer millions of people suffering from the disease worldwide a potent solution by preserving joint function, reducing symptoms, and preventing the need for invasive surgeries.

References

[1] D.J. Hunter, L. March, M. Chew, Osteoarthritis in 2020 and beyond: a Lancet Commission, Lancet 396(10264) (2020) 1711-1712.

[2] H. Long, Q. Liu, H. Yin, K. Wang, N. Diao, Y. Zhang, J. Lin, A. Guo, Prevalence Trends of Site-Specific Osteoarthritis From 1990 to 2019: Findings From the Global Burden of Disease Study 2019, Arthritis & rheumatology 74(7) (2022) 1172-1183.

[3] W. Zhang, H. Ouyang, C.R. Dass, J. Xu, Current research on pharmacologic and regenerative therapies for osteoarthritis, Bone Research 4(1) (2016) 15040.

[4] G.L. Matthews, D.J. Hunter, Emerging drugs for osteoarthritis, Expert opinion on emerging drugs 16(3) (2011) 479-491.

[5] A. Latourte, M. Kloppenburg, P. Richette, Emerging pharmaceutical therapies for osteoarthritis, Nat. Rev. Rheumatol. 16(12) (2020) 673-688.

[6] K. Yudoh, T. Nguyen v, H. Nakamura, K. Hongo-Masuko, T. Kato, K. Nishioka, Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function, Arthritis research & therapy 7(2) (2005) R380-91.

[7] F.J. Blanco, I. Rego, C. Ruiz-Romero, The role of mitochondria in osteoarthritis, Nature Reviews Rheumatology 7(3) (2011) 161-169.

[8] Y.E. Henrotin, P. Bruckner, J.P. Pujol, The role of reactive oxygen species in homeostasis and degradation of cartilage, Osteoarthritis and cartilage 11(10) (2003) 747-55.

[9] M. Koike, H. Nojiri, Y. Ozawa, K. Watanabe, Y. Muramatsu, H. Kaneko, D. Morikawa, K. Kobayashi, Y. Saita, T. Sasho, T. Shirasawa, K. Yokote, K. Kaneko, T. Shimizu, Mechanical overloading causes mitochondrial superoxide and SOD2 imbalance in chondrocytes resulting in cartilage degeneration, Scientific reports 5(1) (2015) 11722.

[10] M.Z. Miao, Q.P. Su, Y. Cui, E.M. Bahnson, G. Li, M. Wang, Y. Yang, J.A. Collins, D. Wu, Q. Gu, S. Chubinskaya, B.O. Diekman, K.M. Yamada, R.F. Loeser, Redox-active endosomes mediate α5β1 integrin signaling and promote chondrocyte matrix metalloproteinase production in osteoarthritis, Science signaling 16(809) (2023) eadf8299.

[11] M.L. Tiku, R. Shah, G.T. Allison, Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation. Possible role in cartilage aging and the pathogenesis of osteoarthritis, The Journal of biological chemistry 275(26) (2000) 20069-76.

[12] C. Rathakrishnan, K. Tiku, A. Raghavan, M.L. Tiku, Release of oxygen radicals by articular chondrocytes: a study of luminol-dependent chemiluminescence and hydrogen peroxide secretion, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 7(10) (1992) 1139-48.

[13] Y. Henrotin, G. Deby-Dupont, C. Deby, M. De Bruyn, M. Lamy, P. Franchimont, Production of active oxygen species by isolated human chondrocytes, British journal of rheumatology 32(7) (1993) 562-7.

[14] C.L. Fattman, L.M. Schaefer, T.D. Oury, Extracellular superoxide dismutase in biology and medicine, Free radical biology & medicine 35(3) (2003) 236-56.

[15] M.Y. Ansari, N.M. Khan, I. Ahmad, T.M. Haqqi, Parkin clearance of dysfunctional mitochondria regulates ROS levels and increases survival of human chondrocytes, Osteoarthritis and cartilage (2017).

[16] H.J. Faust, H. Zhang, J. Han, M.T. Wolf, O.H. Jeon, K. Sadtler, A.N. Peña, L. Chung, D.R. Maestas, Jr., A.J. Tam, D.M. Pardoll, J. Campisi, F. Housseau, D. Zhou, C.O. Bingham, III, J.H. Elisseeff, IL-17 and immunologically induced senescence regulate response to injury in osteoarthritis, The Journal of clinical investigation 130(10) (2020) 5493-5507.

[17] C. Vinatier, E. Domínguez, J. Guicheux, B. Caramés, Role of the Inflammation-Autophagy-Senescence Integrative Network in Osteoarthritis, Frontiers in physiology 9(706) (2018) 1-25.

[18] J. Ghosh, C.E. Myers, Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells, Proceedings of the National Academy of Sciences 95(22) (1998) 13182-13187.

[19] I.M. Avis, M. Jett, T. Boyle, M.D. Vos, T. Moody, A.M. Treston, A. Martínez, J.L. Mulshine, Growth control of lung cancer by interruption of 5-lipoxygenase-mediated growth factor signaling, The Journal of clinical investigation 97(3) (1996) 806-813.

[20] T.W. Friedlander, V.K. Weinberg, Y. Huang, J.T. Mi, C.G. Formaker, E.J. Small, A.L. Harzstark, A.M. Lin, L. Fong, C.J. Ryan, A phase II study of insulin-like growth factor receptor inhibition with nordihydroguaiaretic acid in men with non-metastatic hormone-sensitive prostate cancer, Oncology reports 27(1) (2012) 3-9.

[21] G. Manda, A.I. Rojo, E. Martínez-Klimova, J. Pedraza-Chaverri, A. Cuadrado, Nordihydroguaiaretic Acid: From Herbal Medicine to Clinical Development for Cancer and Chronic Diseases, Frontiers in pharmacology 11(151) (2020).

[22] E. Floriano-Sánchez, C. Villanueva, O.N. Medina-Campos, D. Rocha, D.J. Sánchez-González, N. Cárdenas-Rodríguez, J. Pedraza-Chaverrí, Nordihydroguaiaretic acid is a potent in vitro scavenger of peroxynitrite, singlet oxygen, hydroxyl radical, superoxide anion and hypochlorous acid and prevents in vivo ozone-induced tyrosine nitration in lungs, Free Radic Res 40(5) (2006) 523-33.

[23] T. Tezil, M. Chamoli, C.-P. Ng, R.P. Simon, V.J. Butler, M. Jung, J. Andersen, A.W. Kao, E. Verdin, Lifespan-increasing drug nordihydroguaiaretic acid inhibits p300 and activates autophagy, npj Aging and Mechanisms of Disease 5(1) (2019) 7.

[24] S. Guzmán-Beltrán, S. Espada, M. Orozco-Ibarra, J. Pedraza-Chaverri, A. Cuadrado, Nordihydroguaiaretic acid activates the antioxidant pathway Nrf2/HO-1 and protects cerebellar granule neurons against oxidative stress, Neuroscience letters 447(2-3) (2008) 167-171.

[25] M.K. Lotz, B. Carames, Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA, Nature reviews. Rheumatology 7(10) (2011) 579-87.

[26] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451(7182) (2008) 1069-75.

[27] B. Carames, N. Taniguchi, S. Otsuki, F.J. Blanco, M. Lotz, Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis, Arthritis and rheumatism 62(3) (2010) 791-801.

[28] H. Sasaki, K. Takayama, T. Matsushita, K. Ishida, S. Kubo, T. Matsumoto, N. Fujita, S. Oka, M. Kurosaka, R. Kuroda, Autophagy modulates osteoarthritis-related gene expression in human chondrocytes, Arthritis and rheumatism 64(6) (2012) 1920-8.

[29] J.K. Meckes, B. Carames, M. Olmer, W.B. Kiosses, S.P. Grogan, M.K. Lotz, D.D. D'Lima, Compromised autophagy precedes meniscus degeneration and cartilage damage in mice, Osteoarthritis and cartilage 25(11) (2017) 1880-1889.

[30] B. Carames, A. Hasegawa, N. Taniguchi, S. Miyaki, F.J. Blanco, M. Lotz, Autophagy activation by rapamycin reduces severity of experimental osteoarthritis, Annals of the rheumatic diseases 71(4) (2012) 575-81.

[31] Y. Zhang, F. Vasheghani, Y.H. Li, M. Blati, K. Simeone, H. Fahmi, B. Lussier, P. Roughley, D. Lagares, J.P. Pelletier, J. Martel-Pelletier, M. Kapoor, Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis, Annals of the rheumatic diseases 74(7) (2015) 1432-40.

[32] U. Nogueira-Recalde, I. Lorenzo-Gomez, F.J. Blanco, M.I. Loza, D. Grassi, V. Shirinsky, I. Shirinsky, M. Lotz, P.D. Robbins, E. Dominguez, B. Carames, Fibrates as drugs with senolytic and autophagic activity for osteoarthritis therapy, EBioMedicine 45 (2019) 588-605.

[33] K.M. Dhanabalan, A.A. Dravid, S. Agarwal, R.K. Sharath, A.K. Padmanabhan, R. Agarwal, Intra-articular injection of rapamycin microparticles prevent senescence and effectively treat osteoarthritis, Bioeng Transl Med 8(1) (2023) e10298.

[34] K.M. Dhanabalan, V.K. Gupta, R. Agarwal, Rapamycin-PLGA microparticles prevent senescence, sustain cartilage matrix production under stress and exhibit prolonged retention in mouse joints, Biomater Sci 8(15) (2020) 4308-4321.

[35] G. Filomeni, D. De Zio, F. Cecconi, Oxidative stress and autophagy: the clash between damage and metabolic needs, Cell Death & Differentiation 22(3) (2015) 377-388.

[36] H. Fuhrmann-Stroissnigg, Y.Y. Ling, J. Zhao, S.J. McGowan, Y. Zhu, R.W. Brooks, D. Grassi, S.Q. Gregg, J.L. Stripay, A. Dorronsoro, L. Corbo, P. Tang, C. Bukata, N. Ring, M. Giacca, X. Li, T. Tchkonia, J.L. Kirkland, L.J. Niedernhofer, P.D. Robbins, Identification of HSP90 inhibitors as a novel class of senolytics, Nature communications 8(1) (2017) 422.

[37] I.H. Lee, T. Finkel, Regulation of autophagy by the p300 acetyltransferase, The Journal of biological chemistry 284(10) (2009) 6322-8.

[38] S.M. Son, S.J. Park, E. Stamatakou, M. Vicinanza, F.M. Menzies, D.C. Rubinsztein, Leucine regulates autophagy via acetylation of the mTORC1 component raptor, Nature communications 11(1) (2020) 3148.

[39] S.M. Son, S.J. Park, H. Lee, F. Siddiqi, J.E. Lee, F.M. Menzies, D.C. Rubinsztein, Leucine Signals to mTORC1 via Its Metabolite Acetyl-Coenzyme A, Cell Metab 29(1) (2019) 192-201.e7.

[40] A. Bánréti, M. Sass, Y. Graba, The emerging role of acetylation in the regulation of autophagy, Autophagy 9(6) (2013) 819-29.

[41] M. Del Carlo, D. Schwartz, E.A. Erickson, R.F. Loeser, Endogenous production of reactive oxygen species is required for stimulation of human articular chondrocyte matrix metalloproteinase production by fibronectin fragments, Free Radical Biology and Medicine 42(9) (2007) 1350-1358.

[42] E.-H. Hong, S.-J. Lee, J.-S. Kim, K.-H. Lee, H.-D. Um, J.-H. Kim, S.-J. Kim, J.-I. Kim, S.-G. Hwang, Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase, Journal of Biological Chemistry 285(2) (2010) 1283-1295.

[43] X. Chevalier, P. Goupille, A.D. Beaulieu, F.X. Burch, W.G. Bensen, T. Conrozier, D. Loeuille, A.J. Kivitz, D. Silver, B.E. Appleton, Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study, Arthritis and rheumatism 61(3) (2009) 344-52.

[44] M.A. Karsdal, M. Michaelis, C. Ladel, A.S. Siebuhr, A.R. Bihlet, J.R. Andersen, H. Guehring, C. Christiansen, A.C. Bay-Jensen, V.B. Kraus, Disease-modifying treatments for osteoarthritis (DMOADs) of the knee and hip: lessons learned from failures and opportunities for the future, Osteoarthritis and cartilage 24(12) (2016) 2013-2021.

[45] E.F. Ekman, J.S. Gimbel, A.E. Bello, M.D. Smith, D.S. Keller, K.M. Annis, M.T. Brown, C.R. West, K.M. Verburg, Efficacy and safety of intravenous tanezumab for the symptomatic treatment of osteoarthritis: 2 randomized controlled trials versus naproxen, The Journal of rheumatology 41(11) (2014) 2249-2259.

[46] O.H. Jeon, C. Kim, R.M. Laberge, M. Demaria, S. Rathod, A.P. Vasserot, J.W. Chung, D.H. Kim, Y. Poon, N. David, D.J. Baker, J.M. van Deursen, J. Campisi, J.H. Elisseeff, Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment, Nat. Med. 23(6) (2017) 775-781.

[47] C.H. Evans, V.B. Kraus, L.A. Setton, Progress in intra-articular therapy, Nat. Rev. Rheumatol. 10(1) (2014) 11-22.

[48] G.S.M. John, V.K. Vuttaradhi, S. Takeuchi, R.S. Pitani, G. Venkatraman, S.K. Rayala, Facile synthesis and nanoscale features of a nanostructured nordihydroguaiaretic acid analog for therapeutic applications, Journal of Nanobiotechnology 18(1) (2020) 74.

[49] T.Y. Yang, J.P. Chen, K.W. Ku, S.H. Fu, B.R. Hsu, Survival prolongation of microencapsulated allogeneic islet by nanosized nordihydroguaiaretic acid, Transplantation proceedings 37(4) (2005) 1828-9.

[50] F. Debacq-Chainiaux, J.D. Erusalimsky, J. Campisi, O. Toussaint, Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo, Nat Protoc 4(12) (2009) 1798-806.

[51] F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A. Le Breton, V. Preat, PLGA-based nanoparticles: an overview of biomedical applications, Journal of controlled release : official journal of the Controlled Release Society 161(2) (2012) 505-22.

[52] H.K. Makadia, S.J. Siegel, Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier, Polymers 3(3) (2011) 1377-1397.

[53] A. Singh, R. Agarwal, C.A. Diaz-Ruiz, N.J. Willett, P. Wang, L.A. Lee, Q. Wang, R.E. Guldberg, A.J. Garcia, Nanoengineered particles for enhanced intra-articular retention and delivery of proteins, Adv Healthc Mater 3(10) (2014) 1562-7, 1525.

[54] K.V. Greco, A.J. Iqbal, L. Rattazzi, G. Nalesso, N. Moradi-Bidhendi, A.R. Moore, M.B. Goldring, F. Dell'Accio, M. Perretti, High density micromass cultures of a human chondrocyte cell line: a reliable assay system to reveal the modulatory functions of pharmacological agents, Biochemical pharmacology 82(12) (2011) 1919-29.

[55] A.G. Juby, K. Skeith, P. Davis, Patients' awareness, utilization, and satisfaction with treatment modalities for the management of their osteoarthritis, Clinical rheumatology 24(5) (2005) 535-538.

[56] H. Fang, L. Huang, I. Welch, C. Norley, D.W. Holdsworth, F. Beier, D. Cai, Early Changes of Articular Cartilage and Subchondral Bone in The DMM Mouse Model of Osteoarthritis, Scientific reports 8(1) (2018) 2855.

[57] M. Hou, Y. Zhang, X. Zhou, T. Liu, H. Yang, X. Chen, F. He, X. Zhu, Kartogenin prevents cartilage degradation and alleviates osteoarthritis progression in mice via the miR-146a/NRF2 axis, Cell Death & Disease 12(5) (2021) 483.

[58] J.P. Pelletier, J.P. Caron, C. Evans, P.D. Robbins, H.I. Georgescu, D. Jovanovic, J.C. Fernandes, J. Martel-Pelletier, In vivo suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy, Arthritis & Rheumatism: Official Journal of the American College of Rheumatology 40(6) (1997) 1012-1019.

[59] G.S. Mala John, S. Takeuchi, G. Venkatraman, S.K. Rayala, Nordihydroguaiaretic Acid in Therapeutics: Beneficial to Toxicity Profiles and the Search for its Analogs, Current cancer drug targets 20(2) (2020) 86-103.

[60] E.H. Hong, S.J. Lee, J.S. Kim, K.H. Lee, H.D. Um, J.H. Kim, S.J. Kim, J.I. Kim, S.G. Hwang, Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase, The Journal of biological chemistry 285(2) (2010) 1283-95.

[61] A.P. Evan, K.D. Gardner, Nephron obstruction in nordihydroguaiaretic acid-induced renal cystic disease, Kidney International 15(1) (1979) 7-19.

[62] T. Aigner, A. Sachse, P.M. Gebhard, H.I. Roach, Osteoarthritis: pathobiology-targets and ways for therapeutic intervention, Advanced drug delivery reviews 58(2) (2006) 128-49.

[63] J. Martel-Pelletier, A.J. Barr, F.M. Cicuttini, P.G. Conaghan, C. Cooper, M.B. Goldring, S.R. Goldring, G. Jones, A.J. Teichtahl, J.P. Pelletier, Osteoarthritis, Nature reviews. Disease primers 2 (2016) 16072.

[64] M. Arra, G. Swarnkar, K. Ke, J.E. Otero, J. Ying, X. Duan, T. Maruyama, M.F. Rai, R.J. O'Keefe, G. Mbalaviele, J. Shen, Y. Abu-Amer, LDHA-mediated ROS generation in chondrocytes is a potential therapeutic target for osteoarthritis, Nature communications 11(1) (2020) 3427.

[65] D.M. Findlay, Vascular pathology and osteoarthritis, Rheumatology 46(12) (2007) 1763-1768.

[66] T. Malinin, E.A. Ouellette, Articular cartilage nutrition is mediated by subchondral bone: a long-term autograft study in baboons, Osteoarthritis and cartilage 8(6) (2000) 483-491.

[67] K. Chen, Y. Yan, C. Li, J. Yuan, F. Wang, P. Huang, N. Qian, J. Qi, H. Zhou, Q. Zhou, L. Deng, C. He, L. Guo, Increased 15-lipoxygenase-1 expression in chondrocytes contributes to the pathogenesis of osteoarthritis, Cell Death & Disease 8(10) (2017) e3109-e3109.

[68] B. Carames, N. Taniguchi, D. Seino, F.J. Blanco, D. D'Lima, M. Lotz, Mechanical injury suppresses autophagy regulators and pharmacologic activation of autophagy results in chondroprotection, Arthritis and rheumatism 64(4) (2012) 1182-92.

[69] J.E. Blecha, M.O. Anderson, J.M. Chow, C.C. Guevarra, C. Pender, C. Penaranda, M. Zavodovskaya, J.F. Youngren, C.E. Berkman, Inhibition of IGF-1R and lipoxygenase by nordihydroguaiaretic acid (NDGA) analogs, Bioorganic & medicinal chemistry letters 17(14) (2007) 4026-4029.

[70] S.Y. Kim, T.-B. Kim, K.-a. Moon, T.J. Kim, D. Shin, Y.S. Cho, H.-B. Moon, K.-Y. Lee, Regulation of pro-inflammatory responses by lipoxygenases via intracellular reactive oxygen species in vitro and in vivo, Experimental & Molecular Medicine 40(4) (2008) 461-476.

[71] A. Mobasheri, C. Matta, R. Zakany, G. Musumeci, Chondrosenescence: definition, hallmarks and potential role in the pathogenesis of osteoarthritis, Maturitas 80(3) (2015) 237-44.

[72] M.J. Daniels, J.B. Nourse, H. Kim, V. Sainati, M. Schiavina, M.G. Murrali, B. Pan, J.J. Ferrie, C.M. Haney, R. Moons, N.S. Gould, A. Natalello, R. Grandori, F. Sobott, E.J. Petersson, E. Rhoades, R. Pierattelli, I. Felli, V.N. Uversky, K.A. Caldwell, G.A. Caldwell, E.S. Krol, H. Ischiropoulos, Cyclized NDGA modifies dynamic α-synuclein monomers preventing aggregation and toxicity, Scientific reports 9(1) (2019) 2937.

Declaration

We hereby declare that all the details mentioned in this document are authentic and accurate.

Date: 27-08-2024

Place: Bengaluru

Nominator

Signature:

Name: Dr. Rachit Agarwal

Prof. Rachit Agarwal
Associate Professor
Department of Bioengineering
Indian Institute of Science
Bengaluru-560012

Applicant

Signature:

Name: Bhagyashree Padhan