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Cannabidiol-loaded poly lactic-co-glycolic acid nanoparticles with improved bioavailability as a potential for osteoarthritis therapeutic

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Research Article

Keywords:

Posted Date: November 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2304218/v1

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// Abstract

Cannabidiol (CBD) is a non-intoxicating cannabinoid from cannabis sativa that has demonstrated efficacious against inflammation, which can be considered as a potential drug for arthritis treatment. However, the poor solubility and low bioavailability limit its clinical application. Here, we report an effective strategy to fabricate CBD-loaded poly lactic-co-glycolic acid nanoparticles (CBD-PLGA-NPs). The CBD-PLGA-NPs exhibited a spherical morphology and an average diameter of 238 nm. CBD was sustained release from CBD-PLGA-NPs, which improved the bioavailability of CBD. Primary chondrocytes from rat pups were isolated, and LPS was used to induce inflammation in vitro to simulate osteoarthritis (OA). The CBD-PLGA-NPs effectively protect the damage of LPS to cell viability. What's more, according to the results of CCK-8 assay, hematoxylin-eosin staining, safranin O staining, immunofluorescence staining, and real-time polymerase chain reaction assay, we observed that CBD-PLGA-NPs significantly suppressed LPS-induced primary rat chondrocyte expression of inflammatory cytokines, including interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and matrix metalloproteinase 13 (MMP-13). Remarkably, CBD-PLGA-NPs also showed better therapeutic effects of inhibiting the degradation of the extracellular matrix of chondrocytes than equivalent CBD solution. In general, the fabrication CBD-PLGA-NPs showed good protection of primary chondrocytes in vitro and is a promising system for osteoarthritis treatment.

Significance of the study

Cannabidiol (CBD) is a non-intoxicating cannabinoid from cannabis sativa that has demonstrated efficacious against inflammation, which can be considered as a potential drug for arthritis treatment. In order to improve the poor solubility and low bioavailability of CBD, we described the development of simple and efficient CBD-loaded nanoparticles (CBD-PLGA-NPs) for treating LPS-induced primary chondrocytes of rat pups damaged. The fabricated CBD-PLGA-NPs could effectively enhance the chondroprotective effects of CBD by inhibiting the expression of inflammatory factors, increasing cellularity, and improving structural changes, which can be regarded as a potential system to treat OA.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative disease of the whole joint that involves structural changes in the clear articular cartilage, subchondral bone, ligaments, joint capsule, synovium, and muscles around the joint. About 10% of the population suffers from symptomatic knee OA at the age of 60. Oxidative stress has been shown to be related to the pathogenesis of osteoarthritis and has become an important therapeutic target. Reducing oxidative stress in cartilage that caused by inflammation can improve chondrocyte viability, which would contribute to the treatment of osteoarthritis. Hormones and non-steroidal anti-inflammatory drugs are commonly used in the clinical treatment of osteoarthritis. Yet they can only relieve the symptoms, and long-term use of such drugs will cause significant side effects, such as suppression of platelet aggregation, erosions, and ulcerations in upper gastrointestinal tract mucosa, so as it is necessary to explore more effective drugs or materials for the treatment of OA. The intra-

articular injection is an effective treatment for OA by delivering the drug to the site of action, thereby minimizing the systemic toxic effects of the drug. However, the synovial membrane of the joint is easily cleared by drugs, new nano-sustained-release drug delivery systems are urgently needed for the treatment of OA .

A non-psychoactive component of cannabis, CBD, does not cause physiological dependence and is well tolerated. Studies have shown that CBD has certain therapeutic effects on nervous system diseases, epilepsy, tumors, inflammation, liver injury, diabetes, pain, and other diseases. In addition, the abilities to inhibit TNF-α and modulate the immune system of CBD were helpful for the treatment of collagen-induced rheumatoid arthritis. In a lipopolysaccharide-induced model of microglial inflammation, CBD treated inflammation by inhibiting ROS/NF-κb dependent signaling and glucose-dependent NADPH production. For musculoskeletal disease, systemic administration of CBD effectively suppressed the progression of collagen-induced arthritis by reducing inflammatory cytokine production. Since the progression of OA is closely related to the production of inflammatory factors, CBD may show potential therapeutic effects in OA. Taking these pharmacological properties into consideration, CBD represents an attractive therapeutic option for OA. Unfortunately, its bioavailability has been reported to be extremely low when given orally to dogs, presumably due to a high first-pass effect through the liver. Improving the bioavailability of CBD is important for the clinical application of CBD.

PLGA-based drug products have been approved by the FDA and European Medicine Agency for parenteral administration as drug delivery systems. The utilization of PLGA to fabricate nano/microparticles presented a broad application in disease treatment. PLGA has well-described formulations and methods of production that adapt to various types of drugs and contrast agents, such as hydrophilic or hydrophobic small molecules and macromolecules. The drug and contrast agent can be protected by PLGA which avoids degradation. The surface properties of PLGA can be modified to provide "stealth" and/or better interaction with biological materials. Many targeting and imaging moieties conjugated with PLGA particles to enhance their binding affinity and specificity, and achieve amplifying signals at the target region. Moreover, The PLGA-based nanoparticles can be modified to target specific organs or cells for effectively delivering imaging labels, prolonging plasma half-lives, enhancing stability, improving targeting efficiency, and reducing non-specific binding. PLGA has been widely used in drug delivery systems due to its biocompatible and biodegradable properties, while the biocompatible and biodegradable are essential parameters are considered of polymer. On the other hand, the degradation products of PLGA, lactic and glycolic acid, which are natural metabolites of the human body, indicating PLGA was safe enough used in biomaterials fabrication. Considering the multitudinous advantages of PLGA acting as a biomaterial, the bioavailability of CBD may be improved that modified with PLGA, which is conducive to its further application.

Herein, PLGA was employed to carry CBD and fabricated to nano drug delivery system (CBD-PLGA-NPs). The CBD-PLGA-NPs presented an average diameter of 238 nm, high encapsulation efficiency, and sustained CBD release. Moreover, CBD-PLGA-NPs significantly reduced primary chondrocytes of rat pups secreted IL-1β, IL-6, TNF-α, and MMP13 that induced by LPS and showed protection for the chondrocyte

activity. The CBD-PLGA-NPs achieved the goal of high efficiency in clearing inflammatory factors of chondrocytes, which may be regarded as a potential system for OA treatment.

2. Materials And Methods

2.1 Materials

Cannabidiol (SolarBio, Beijing, China), dichloromethane (SolarBio, Beijing, China), PLGA(SolarBio, Beijing, China), PVA (SolarBio, Beijing, China), CCK-8 reagent (SolarBio, Beijing, China), Total RNA extraction kit (SolarBio, Beijing, China), Hematoxylin-Eosin (H&E) staining kit (SolarBio, Beijing, China), Safranin O staining kit (SolarBio, Beijing, China), IL-6 primary antibody (Boster, Wuhan, China), MMP13 primary antibody (Boster, Wuhan, China), collagenase II (Solarbio, Beijing, China), cyanine/streptomycin, FITC immunofluorescence antibody (Bodder, USA), Calcein-am/Ethd-I staining reagent (Sigma, USA), DAPI (SolarBio, Beijing, China), Penicillomycin (SolarBio, Beijing, China), Fetal Bovine Serum (Sijiqing, Zhejiang, China), DMEM Media (Solarbio, Beijing, China), LPS (SolarBio, Beijing, China).

2.2 Instruments

Shaker (New Brunswick Science), Ultrasonic Cleaner (Allied Cleaning Equipment Company), Transmission Electron Microscopy (Bruker, Germany), 6-well plate (Corning, USA), 24-well plate (Corning, USA), 96-well plate (Corning, USA), Cell culture incubator (Forma, USA), fluorescence quantitative PCR apparatus and enzyme label apparatus (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Preparation and Characterization of CBD-PLGA-NPs

Two different types of nanoparticles were fabricated for the study: (i) PLGA nanoparticles, (ii) Cannabidiol-encapsulated PLGA nanoparticles (CBD-PLGA-NPs). For fabricating CBD encapsulated nanoparticles, 10 mg CBD and 30 mg PLGA was dissolved in 1 mL dichloromethane and dispersed by ultrasonic to form an oil phase. The oil phase was added drop by drop into 2 mL 1% polyvinyl alcohol (PVA) solvent with a 1 mL syringe and stirred in a magnetic agitator for 30 minutes. The ultrasonic probe was used to disperse the oil phase on the ice at 4 ^{O}C to form colostrum. The colostrum was added into 6 mL 1% PVA solution, magnetically stirred for 4 hours, and volatilized organic solvents were washed with double steam water, centrifuged three times, lyophilized, and stored in a storage container at 4 ^{O}C for retention. PLGA nanoparticles without drug loading were prepared by the same method.

2.4 CBD-PLGA-NPs size detection

The freeze-dried nanoparticles were dissolved in double-steaming water, and the samples were evenly dispersed by ultrasound for about 30 s. The fully dispersed samples were placed in a 2 mL colorimetric dish, and the particle size of the nanoparticles was measured by a Malvin particle size analyzer. The experiment was repeated three times for each sample to determine the average particle size and particle size distribution.

2.5 Transmission electron microscopy (TEM) analysis

Firstly, to study the solubilization and stability of CBD-PLGA-NPs, 5 mg PLGA-NPs and 5 mg CBD-PLGA-NPs were dissolved in 1 mL of ultrapure water, respectively, followed by ultrasound for 15 min and the samples were added to the carbon net and dried at room temperature, then photographed for observation. The morphology of PLGA-NPs and CBD-PLGA-NPs was determined by using TEM (Bruker, Germany).

2.6 Release characteristics of CBD from CBD-PLGA-NPs

The chromatographic conditions were as follows: ZORBAX300SB-C18column (4.6 mm×250 mm), analysis wavelength was set at 220 nm, the mobile phase was acetonitrile and methanol (65:35, V/V), the flow rate was 2.0 mL/min, and sample volume was 20 μL. The determination of standard curve: The analytical balance was used to accurately the concentration of CBD (1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg/mL).

The release behavior of CBD from the CBD-PLGA-NPs was monitored at 37°C.8 mg CBD-PLGA-NPs were placed in a dialysis bag (MW3500, SolarBio), placed in 8 mL PBS solution containing 1% TW (pH = 7.4), and placed in a thermostatic gas bath vibrator at 37 ^{o}C (100 rpm/min). 1 mL sample was withdrawn at predetermined time intervals from each dissolution medium and was replenished to sustain the condition of the sink. The released CBD concentration was analyzed by using HPLC. All the studies were performed three times and the mean value was calculated .

2.7 Determination of CBD loading rate and encapsulation rate

1 mg CBD-PLGA-NPs were dissolved in 1ml dichloromethane and 4ml methanol, and the nanoparticles were destroyed by repeated vortices using a vortex instrument to fully release the drug. Centrifugation was conducted at 10000 rpm/min, and the supernatants were collected and estimated using High-Pressure Liquid Chromatography (HPLC). The percentage drug loading and encapsulation efficiency of the nanoparticles were calculated using the following equation:

$$drugloading = \frac{Amountofcannabidiolencapsulated}{Totalmassofmicrospheres} * 100 \text{ (a)}$$

$$EncapsulationEfficiency = \frac{AmountofCannabidiolencapsulated}{TotalamountofCannabidioladded} * 100 \text{ (b)}$$

2.8 Cytotoxicity studies

Primary chondrocytes were harvested from a 5-day-old Sprague Dawley rat. Curtly, cartilage was obtained from the joints of rats' limbs and cut into small pieces nearly 1 mm^3 size. The cartilage debris was digested by 0.25% trypsin at 37°C for 30 min, and 0.2% type II collagenase (Solarbio, Beijing, China) for 4 h. Cells were collected by centrifugation (1000 rpm, 5 min) and cultured in Modified Eagle's medium (Solarbio, Beijing, China) containing 10% (v/v) fetal bovine serum (Sijiqing, Zhejiang, China) and 1% (v/v) penicillin/streptomycin (Solarbio, Beijing, China) in an incubator with 5% CO_2 at 37°C. Cells were passaged when reaching nearly 80%-90% till the second generation for further research.

The viability of CBD-PLGA-NPs on the proliferation of LPS-induced chondrocytes was measured by CCK-8 assay. (Keygen, Nanjing, China). Briefly, the chondrocytes were seeded in a 96-well plate at a density of 5000 cells overnight. Thereafter, various concentration of CBD-PLGA-NPs was added and further incubated for 48 h. After treatment, 10 µL of CCK-8 were added to each well and cultivated for 4 h. Finally, the absorbance at 450 nm was measured using a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer).

2.9 Live/dead tests

The Live/dead cells assay was performed using a live/dead viability assay kit (Invitrogen, USA). The cells were washed with PBS and incubated with the solution containing 2 µM of Calcein AM and 4 µM of Ethidium homodimer-1 for 30 min at room temperature in the dark. Then the cells were washed with PBS before being observed using a fluorescent microscope (Olympus BX53, Tokyo, Japan). As described in the manufacturer's protocol, live cells were stained green and dead cells were stained red.

2.10 qRT-PCR detection

RNA separation kits (Magen, China) were used to extract total RNA. The reverse transcription is done by a reverse transcription kit (Fermentas Company, USA) .

All qRT-PCR reactions were performed by a light Cycle 96 system (Roche, Switzerland) for 10 min at 95°C, followed by 40 cycles with 10 s duration at 95°C and then 60 s duration at 60°C. Each experiment was measured in triplicate, and $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression of each sample. The primers were used as follows in the Table 1.

Table 1
Primers for RT-PCR performance

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GADPH	CACGACATACTCAGCACCAG	TCCAGTATGACTCTACCCACG
IL-1β	GCACAGTTCCCCAACTGGTA	GGAGACTGCCCATTCTCGAC
IL-6	ACAAGTCCGGAGAGGAGACT	ACAGTGCATCATCGCTGTTC
TNF-α	AACCAAGATGTGGAGTGCCTGATG	GGGCTCCACATTGCAGAGAA
MMP13	TCCTCTTGGTGGCTGACTCTTCC	GGCATGACTCTCACAATGCG
2.11 Hematoxylin and Eosin (H&E) staining		

For *in vitro* study, the cells in all groups were fixed with 95% alcohol for 30 min. After washing with PBS, the slides were fixed and cleaned, dyed with hematoxylin for 2–3 min, washed with PBS, soaked with Eosin for 1min, and washed with PBS. After the slides were dried, the slides were sealed with neutral resin, and cell morphology was observed under a microscope and photographed.

2.12 Safranin O staining assays

For *in vitro* study, Safranin O staining was used to evaluate the deposition of glycosaminoglycans (GAGs). Cells in all groups were fixed with 95% alcohol for 30 min. After washing with PBS, the cell slides were soaked with Safranin O dye for 15 min, then cleaned with PBS. After natural drying, the slides were sealed with neutral resin. Cell morphology was observed under a microscope and photos were taken.

2.13 Immunofluorescence staining assay

The chondrocytes with a density of 1×10^4 cells/mL were cultured in the 24-well plates with sterile cover glasses, treated with LPS (5 nM) for 12 h, and then co-cultured with 1.5 mg/mL of the above CBD-PLGA-NPs solutions. After co-culturing for 12 h, the chondrocytes were fixed with 4% paraformaldehyde for 10 min, and then permeabilized with 0.1% Triton X-100 (Aladdin Bio-Chem Technology Co, Ltd., Shanghai, China) and blocked with 3% bovine serum albumin/phosphate-buffered saline (BSA/PBS, Aladdin Bio-Chem Technology Co, Ltd, Shanghai, China) at 25 ^{o}C for 15 min and 30 min, respectively. After washing with PBS, the cells were stained against the specific rat primary antibody of MMP-13 and IL-6 (at 1:200 dilution, Boster, Wuhan, China) overnight at 4 ^{o}C. Subsequently, the cells were gently washed with PBS and reacted against the specific Alexa Fluor-coupled secondary anti-rabbit of IgG (1:100 dilution, Boster, Wuhan, China) at 25 ^{o}C for 1 h in darkness. Meanwhile, the cell nuclei were counterstained using 4, 6-diamidino-2-phenyindole dilactate (DAPI, Life Tech, USA) in darkness for 15 min, and the cell actin was labeled by Alexa Fluor 594 phalloidin (Life Tech, USA). Finally, the staining images were captured using a laser scanning confocal microscope (LSM800, ZEISS, Germany), and the expression of MMP-13 and IL-6 was investigated by the Image J software.

2.14 Statistical analysis

All data were reported as mean ± standard deviation of at least three experiments. The two-sided student t-test was used for statistical analysis. P value less than or equal to 0.05 was considered statistically significant.

3. Results

3.1 Fabrication and characterization of CBD-PLGA-NPs

TEM was performed to analyze the morphology of PLGA-NPs and CBD-PLGA-NPs. As shown in Fig. 1A&1B, PLGA-NPs and CBD-PLGA-NPs had regular spherical morphologies with an average diameter of 200 nm. CBD did not much change the morphology of PLGA-NPs, and PLGA-NPs and CBD-PLGA-NPs presented well dispersion. The particle size determination of PLGA-NPs and CBD-PLGA-NPs was reported by Malvin particle size analyzer. The average particle size of PLGA-NPs and CBD-PLGA-NPs were (226.06 ± 5.58) nm and (238.82 ± 1.10) nm, respectively, which is consistent with TEM results.

3.2 Drug release from CBD-PLGA-NPs

The CBD-loading and encapsulation efficiency of CBD-PLGA-NPs were measured using HPLC. The results showed that CBD-PLGA-NPs exhibited high drug-loading and encapsulation efficiency, which were up to 22.1 ± 2.87% and 88.4 ± 11.49%, respectively. The release behavior of CBD from CBD-PLGA-NPs was further tested. CBD concentrations were obtained using an acceptable standard curve (Fig. 2A), and the standard curve formula is as follows: Y = 99093X + 380819, R2 = 0.9961. The good linear relationship between concentration and peak area in various concentration CBD means that CBD released from CBD-PLGA-NPs concentration would be detected accurately. As shown in Fig. 2B, CBD was sustained released in the detection period, which is up to 40 days, indicating that the CBD-PLGA-NPs have the function of long-term slow release.

3.3 Anti-inflammatory Assay

CCK8 assessment was performed to detect the anti-inflammatory activity of CBD-PLGA-NPs. As shown in Fig. 3, after LPS induction, CBD-PLGA-NPs treated cells viability decreased to 67.92% at the concentration of 30 µg/mL, while the viability of the cells was not different compared with the control group at concentrations below. Therefore, 20 µg/mL of CBD-PLGA-NPs were applied for the subsequent experiments.

The live/dead cell staining assay revealed that cell survival was obviously decreased with the stimulation of LPS, as evidenced by more dead cells and less viable cells in the control group (Fig. 4). While the treatment of CBD or CBD-PLGA-NPs showed weaker red fluorescence light than the control group, indicating that the cell survival rate was significantly increased. Particularly, more viable cells were observed from the CBD-PLGA-NPs group, suggesting that CBD-PLGA-NPs can be served as an effective inhibitor against LPS-induced cell dead in chondrocytes.

3.4 Detection of IL-1β, IL-6, TNF-α and MMP-13 gene expression

Gene expression levels of cartilage-specific markers including IL-1β, IL-6, TNF-α, and MMP-13 were analyzed by qRT-PCR to explore the effect of CBD-PLGA-NPs on chondrocytes. The levels of IL-1β, IL-6, TNF-α, and MMP-13 were notably increased by the stimulation of LPS (Fig. 5). CBD-PLGA-NPs remarkably decreased the damage in chondrocytes that were stimulated by LPS. Furthermore, among the CBD and CBD-PLGA-NPs treated groups, most minimized changes in phenotype loss of chondrocytes were shown in CBD-PLGA-NPs group. The expression levels of IL-1β, IL-6, TNF-α, and MMP-13 genes were increased when LPS-induced inflammation compared with the control group ($P < 0.05$). In contrast, the expression levels of IL-1β, IL-6, TNF-α, and MMP-13 genes were significantly decreased after treatment with CBD-PLGA-NPs ($P < 0.05$), which indicated that CBD-PLGA-NPs could down-regulate the expression of inflammation-related genes.

3.5 Hematoxylin and eosin (H&E) staining

H&E staining was used to detect the effect of CBD-PLGA-NPs on the morphology of chondrocytes. The morphology of chondrocytes in the control group all showed the perfect condition. While chondrocytes were transformed into elongated and fibroblast-like ones and lost the typically fusiform-like shape when treated with LPS (Fig. 6). CBD-PLGA-NPs treatment reversed the morphology of LPS-treated chondrocytes to polygonal and round shape. CBD can also recover several chondrocytes. Yet, the recovered ratios were much lower than that CBD-PLGA-NPs treatment group, which indicated that CBD-PLGA-NPs showed better chondrocytes protection function.

3.6 Safranin O staining

To further verify the effect of CBD-PLGA-NPs on the extracellular matrix (ECM) protection of chondrocytes, safranin O staining, which stained the glycosaminoglycan (GAG, a main component of ECM of chondrocytes) was detected. As shown in Fig. 7, LPS treatment group cells showed less red, indicating that LPS caused obvious GAG loss compared with the control group. The GAG of CBD-PLGA-NPs treatment group was similar to the control group, suggesting that CBD-PLGA-NPs had the most significant potent inhibition on GAG loss. What's more, the chondrocyte morphology was similar to those in the control group, which was consistent with H&E staining results.

3.7 IL-6 and MMP-13 protein of chondrocytes analysis

The effect of CBD-PLGA-NPs on the expression of inflammatory factors IL-6 and MMP-13 in chondrocytes was detected by immunofluorescence assay. The secretions of IL-6 and MMP-13 were the features of OA. The results of immunofluorescence staining showed that obvious green fluorescence appeared in the LPS treatment group, indicating the high expression level of IL-6 and MMP-13 in the LPS group. However, the green fluorescence of chondrocytes that were treated with CBD-PLGA-NPs was hardly observed. These results suggested that CBD-PLGA-NPs exhibited a better anti-inflammatory against OA chondrocytes, which would be helpful for OA therapy.

4. Discussion

OA is a chronic degenerative disease, mainly due to the invasion of inflammatory factors and the imbalance of oxidative stress balance. Inhibiting the expression of inflammatory factors is an efficient way to treat OA. CBD has been shown anti-inflammatory properties in previous studies. Thus, it is a promising candidate for treating OA. However, the low solubility and bioavailability limit its further application in OA treatment. Developing a simple and effective drug delivery system that can sustainably release CBD is meaningful for increasing CBD utilization. Takahashi et al. showed that oral administration of a liposome-encapsulated CBD to rats could significantly increase the bioavailability and cellular uptake efficiency of CBD compared to free CBD. The pharmacokinetics in vivo revealed that CBD-entrapped nano-particles demonstrate at least a 9-fold increase in oral bioavailability when compared to CBD administered with piperine as an absorption enhancer. PLGA is widely used as a drug carrier due to its biodegradable and drug control release characteristic. In this study, CBD and PLGA were

used to fabricate CBD nano drug delivery system (CBD-PLGA-NPs) aimed to explore the therapeutic effect of CBD on LPS-induced inflammation of chondrocytes. CBD-PLGA-NPs were synthesized by ultrasonic with uniform particle size and presented slow drug release characteristics. CBD-PLGA-NPs achieved continuous release, which may avoid the CBD rapid clearance in the joint cavity, thus resulting improve CBD treatment effect. Furthermore, the anti-inflammatory activity detection showed that CBD and CBD-PLGA-NPs significantly inhibited the OA development-related gene expression, such as IL-1β, IL-6, TNF-α, and MMP-13 genes. It is worth noting that CBD-PLGA-NPs performed a better inhibition effect than free CBD. These studies demonstrated that CBD-PLGA-NPs could enhance CBD chondroprotective effects against LPS-induced inflammation of rat chondrocytes. The enhancement of chondroprotective effects of CBD encapsulated in PLGA may relate to an increase in CBD bioavailability and stability.

Further detecting the anti-inflammatory of CBD-PLGA-NPs, the results revealed that CBD-PLGA-NPs could reverse hypocellularity in LPS-induced chondrocytes, which was shown better than the free CBD. Reducing cellularity is a characteristic feature of OA cartilage. Recent studies have shown a positive correlation between the degree of severity of OA and chondrocyte loss in both experimentally induced OA in rabbit cartilage and human OA cartilage. Actually, saving the plausible number of cartilage cells in the joint articular structure is important in OA pathology and progression, because chondrocytes are the only component capable of controlling vital activities of the articular cartilage.

As for cartilage tissue engineering, extracellular matrix (ECM) proteins in cartilage are of great significance for the regulation of cell behavior, proliferation, differentiation, and morphogenesis. Earlier studies have reported that LPS has an inhibitory effect on the activity of glyceraldehydes-3 phosphate dehydrogenase in chondrocytes resulting in disruption of glycolysis, hydration of the extracellular matrix, increased extractability as well as reduced quantity and synthesis of proteoglycans, and eventually leads to cell death. In addition to the change of cellularity, the ECM was observed reduced in LPS-treated cells, which suggested that the cells were damaged by LPS. Proteoglycan depletion may be secondary to cell loss due to the OA process. CBD-PLGA-NPs treated cells not only increase cellularity but also efficiently prevent LPS-induced ECM degradation. It is a possibility that CBD-PLGA-NPs penetrate the chondrocytes and stimulates glycosaminoglycan and proteoglycan synthesis. Study has revealed that CBD can stimulate matrix synthesis by restoring glycosaminoglycan synthesis. Histological examination using Mankin scoring showed that intra-articular injection of LPS in rat knee joints induced cartilage structural changes, matrix degradation, and chondrocyte disorganization. What’s more, MMP-13, a key enzyme of cartilage matrix degradation in osteoarthritis, expression was also inhibited.

5. Conclusion

In summary, we described the development of simple and efficient CBD-loaded nanoparticles (CBD-PLGA-NPs) for treating LPS-induced primary chondrocytes of rat pups damaged. The fabricated CBD-PLGA-NPs could effectively enhance the chondroprotective effects of CBD by inhibiting the expression of inflammatory factors, increasing cellularity, and improving structural changes, which can be regarded as a potential system to treat OA.

Declarations

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

Funding:

This study was supported by the National Natural Science Foundation of China (grant no. 81960414), the Guangxi Science and Technology Base and Talent Special Project (grant no. GuikeAD19254003).

Author Contributions:

Conceptualization: Q. W., S. X.; methodology: Q. W., Z. Q., Z. J., S. X. and Y. Z.; validation: Q. W., Z. Q.; formal analysis: Z. J. and Y. Z.; investigation: Z. J., Y. Z., L. Z., Z. Q., Q. W. and S. X.; resources: Q. W. and S. X.; data curation: Y. Z. and Z. Q.; writing-original draft preparation: Z. J. and S. X.; writing-review and editing: Z. J., Y. Z., Z. Q., L. Z., Q. W. and S. X.; visualization: Z. Q., Z. J. and Y. Z.; supervision: Z. Q., Y. Z. and L. Z.; project administration: Z. Q., Q. W., and S. X.; funding acquisition: L. Z., Q. W., S. X.

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Unsectioned Paragraphs

All authors have read and agreed to the published version of the manuscript.