An anti-inflammatory nanoghost for atherosclerosis therapy: a red blood cell based bio-mimetic strategy

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Abstract

Inflammation control is becoming a critical strategy for atherosclerosis management, because inflammation is involved in plaque progression. In this regard, a biomimetic strategy using cell membrane-coated nanoparticles has some promising advantages. In the study, a RBC membrane-based nanoghost containing Glyburide (Glibenclamide) was prepared using an extrusion method. The hydrodynamic size and zeta potential of the nanoghost were changed compared to PLGA nanoparticles. In addition, a nanoghost with a diameter and shell size of 125nm and 8.3nm was obtained based on the TEM measurement. The fabricated nanoghost was not only hemocompatible but also was biocompatible. According to RT-PCR assay, the expression levels of inflammatory genes including NLRP3, IL-1β, IL-18 caspase1, 8 and 9 were decreased. In accordance with in vitro anti-inflammation properties, total foam cells, total surface area in tunica intima and population of CD14 + cells were decreased in the rabbit model of atherosclerosis upon nanoghost treatment, compared to positive control. Furthermore, macrophages in aorta sections exhibited M1 to M2 polarization. In general, the development of Glyburide-loaded nanoghost can be considered as a potential therapeutic for controlling the progression and inflammation of atherosclerotic plaque.

Introduction

Recent advances in biochemical sciences have shown that chronic diseases such as atherosclerosis are mediated by inflammatory responses. Inflammation is a process in which circulating leukocytes and plasma proteins migrate to the site of infection in tissues and are activated to destroy and eliminate invading agents. Inflammation is also the main reaction to dead or damaged cells, as well as the accumulation of abnormal substances in cells and tissues (1). Innate and acquired immune elements, cells or molecules, are involved in the process of acute and chronic inflammation. Among them, neutrophils, monocytes and macrophages have been introduced as key cells, contributing to the expression of inflammatory macromolecules and activation of pathways such as the inflammasome.

The inflammasome is known as a complex of proteins that respond to a wide range of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). It has been reported that this complex includes NOD-like receptors (NLR) such as NLRP3, IPAF and the NLR protein, which recruits the ASC protein, an inflammatory adapter, that binds to caspase-1 and caspase-11, leading to the transcription of pro-inflammatory cytokines and interleukin (IL) genes (IL-1β and IL-18). The inflammasome activation has been established in inflammatory pathways, playing a significant role in the clinical symptoms of many diseases, including diabetes, multiple sclerosis, MS, rheumatoid arthritis, atherosclerosis and others.

Atherosclerosis is defined as the thickening of the vessel walls that can develop in arteries supplying blood to various organs such as the heart, brain and kidneys. According to the reports, it has appeared to be a major cause of heart attack and stroke (2). It is also known as a progressive inflammatory disease, involving the immune system at the site of lipid and plaque accumulation Indeed, studies have shown
innate and acquired immunity at molecular and cellular levels plays an important role in the pathophysiology of the disease(3).

Novel strategies have been focused on treatment of atherosclerosis using anti-inflammatory drugs such as canakinumab to neutralize down-stream secretory interleukin. However, anti-inflammasome agents are introduced as effective and affordable therapeutics for upstream inhibition of inflammasome in some inflammatory diseases like atherosclerosis. In this regard, anti-inflammasome properties of Glyburide, a type 2 diabetes medication, have been recently reported (4).

The potential use of nanoparticles for the delivery of therapeutic agents to cardiovascular defects is considered a promising perspective for the treatment of related diseases (5). In this regard, improvement in the efficacy of therapeutic agents for atherosclerosis treatment is facilitated by the utilization of nanomedicine in the design of nanocarriers. Besides the exploitation of nanotechnology, approaches targeting plaque have improved the efficacy of atherosclerosis management. Particularly, exploiting the homing capability of the blood cell as carriers has been navigated therapeutics to the desired site. This nanocarrier would also be expected to improve the efficacy of drug delivery and overcome the limitation of detection by reticuloendothelial system (RES) and subsequent phagocytosis and fast clearance. Unfortunately, the reduced half-life of the cell-based nanocarriers, due to life dependency, exocytosis and internal hydrolysis of the therapeutic have limited their application. Therefore, cell membrane-coated nanoparticles, called nanoghosts, have been developed to remove the major drawbacks. Taking advantage of the cell membrane-associated adhesion molecule in the shell compartment as well as nanoparticle form in the core compartment, this nanoghost can recognize the target site in a homotypic targeting manner, escape the immune system, increase half-life, penetrate efficiently and provide a sustained delivery system (6, 7).

Different types of biological cell membranes have been used to develop nanoghosts. Among them, red blood cell membrane (RBC) is known as the most abundant cells in the peripheral blood with good compatibility and cells relatively long half-life (120 days). Furthermore, the existence of the CD47 biomarker on the RBC membrane, having a “don’t eat me” message has enabled nanocarriers to escape from the immune system (8). A pathogenic feature of red blood cells (RBC) in an oxidized form has been reported in a plaque lesion. It was reported that binding of RBC membrane to inflammatory chemokines can change its level in atherosclerotic plaque. This RBC incorporated plaque site will undergo phagocytosis (9, 10). It should be mentioned that glycoprotein A as a marker of the RBC membrane has been found in increased plaque lesions, considering its role in accumulation of cholesterol (11).

In this study, we have prepared PLGA nanoparticles, loaded with anti-inflammasome Glyburide and then coated with RBC membrane (Fig. 1). The prepared nanoghost was physiochemically characterized and after ensuring its bio-hemocompatibility, its drug release profile, the potential of the nanoghost in cellular uptake, on inhibition of inflammatory genes and finally preventing plaque formation in the rabbit model of atherosclerosis, was investigated.
Materials and methods

Glyburide was purchased by Keyuan Pharma Co (Shandong, China). PLGA (Mw.38000 – 4000, 50:50) was provided by Sigma Company (Dubai, United Arab Emirates). All reagents and solvents were purchased from Merck Company (Dubai, United Arab Emirates). The phosphate inhibitor was kindly provided by the Kiazist Company (Hamedan, Iran).

RBC separation

The whole fresh blood was kindly gifted from Pooyesh Pathobiology Lab, heparinized and then centrifuged at 5000 rpm for 5 minutes. The plasma was discarded, washed three times with PBS. The cells were lysed in distilled water for 20 minutes and the lysate was centrifuged at 8500 rpm for 30 minutes. The washing procedure was continued until the precipitation of pinky pellet and clear supernatant. The pellet was finally stored at 4°C (12).

Synthesis of PLGA nanoparticles

PLGA nanoparticles were first prepared by emulsification solvent evaporation method. Briefly, PLGA and Glyburide were dissolved in 2 mL of dichloromethane using a vortex to form a homogeneous solution of drug and polymer. The solution was then added slowly to 20 ml of aqueous phase of 1% PVA under probe sonication (MISONIX) at 60 amplitudes for 5 min in an ice bath. The prepared nanoparticle was stirred using a magnetic stirrer for 4 hours at room temperature. The PLGA NPs were collected and washed by centrifugation (Remi, India) at 9000rpm for 15 min to participate nanoparticles(13).

Preparation of RBC membrane-coated NPs

To prepare NG, the lysate of RBC membrane was first homogenized and then extruded through polycarbonate membrane with pore sizes of 400 and 200nm 5 times, sequentially. Each membrane obtained from 1mL of whole blood was mixed with 1mg of Glyburide loaded with PLGA NPs. The mixture was then extruded through the same membrane with a pore size of 200 nm 5 times(14).

Characterization

Size, polydispersity and zeta potential measurements

To characterize the NG, the size, dispersity and zeta potential of particles were measured by zeta sizer (Malvern, Nano ZS90). To measure the actual size of the nanoghost using transmission electron microscopy (TEM), the sample was stained with uranyl acetate dye and then visualized.

Verification of membrane sidedness

To ensure the correct sidedness of the cell membrane when coated on a PLGA nanoparticle, the sialic acid amount was measured. For this, RBC membrane (positive control), PLGA NP (negative control) and NG in equal ratio were separately mixed with 100 units of sialidase (Sigma-Aldrich) to incubate for 3h
followed by centrifugation at 100000 × g for 1.5 h. To determine the sialily groups, a colorimetric assay was conducted at 549nm (15).

**Drug loading estimation**

To measure the Glyburide in unknown solutions, the chromatographic procedure was performed using C18 column (25mm×4.6mm; 5µm), mobile phase of acetonitrile: 0.1 M phosphate buffer (55:45), pH 3 at wavelength of 222 nm. To prepare a loading sample, 2mg of the lyophilized nanoghost was dissolved in the mobile phase. Thereafter, 2 mg of the Glyburide was dissolved in 5 ml of mobile phase to prepare a standard stock solution of 400µg/ml. It was diluted more to obtain a standard solution of 800 ng/mL. Finally, the filtered solutions were injected by auto-sampler (16).

**In vitro drug release**

The release kinetic of NG was investigated by transferring 1mL of nanoghosts suspension in medium of PBS (pH 7.4) into a dialysis bag in a Falcon tube under stirring at 100rpm. The samples were taken at different time intervals, then were replaced with the same volume of PBS. Drug release percentages were determined according to the standard calibration curve by HPLC procedure, like the loading estimation section (17).

**Hemolysis assay**

The whole blood, which was received from 16 Azar clinic (Tehran University) was citrated, centrifuged at 2000 rpm to remove plasma and debris. The RBC pellet was washed 3 times by PBS buffer by centrifugation at 3500 rpm. The precipitated RBC suspension was diluted 3 times. To test hemolytic activity, 200mL of NG suspension with different concentrations, PBS buffer as negative control and Triton X100 as positive control were separately added to 800mL diluted RBC suspension and mixed gently by inversion, shaking at 100rpm for 2h at 37°C. Subsequently, all samples were centrifuged at 8,000g for 2 min, and the absorbance of the supernatants was recorded at 541 nm using (JASCO) UV–vis spectrophotometer (18, 19).

**MTT assay**

The cytotoxicity test for U937 cell line was performed in DMEM medium, supplemented with FBS 5%, and 400µl penicillin- streptomycin. 100µl cell suspension containing 4.4×104 cells/mL was transferred into each well and left for 24h in an incubator at 37°C and 5% CO2 after 24h. Thereafter, 100µl of free drug, nanoparticle and nanoghost in concentrations of 10, 25, 50, 75, 100, 250, and 500µg/mL were separately added to wells and incubated for 48h. After the exposure time, MTT reagent is added, and dissolved by DMSO addition. Absorbance of each well was read at 542nm using a pellet reader. Finally, the cell viability percentages were calculated (20).

**Apoptosis assay**

Besides cell viability, the apoptosis rate was also evaluated. Briefly, the cell suspension containing 2.5-3×105 cells/ml was transferred into each well and left for 48h, in an incubator at 37°C and 5% CO2.
Thereafter, the cells were transferred into each well on a 6-well plate and treated with 250µg/mL NG and NP for 6h. The cells were centrifuged at 5000 rpm for 5min, followed by suspending in 100µL of binding buffer. The suspension was sequentially mixed with 1pL of Annexin V-FITC and PI dyes for 20min. Finally, the apoptosis rate was determined based on flow-cytometry results(21).

Real time PCR

The genes of NLRP3, IL1-β, IL-18, caspase 1, caspase 8 and caspase 9 were amplified by PCR using primers described in table S1. To evaluate the effect of Glyburide on expression level of inflammatory genes, RT-PCR assay was performed so that the total RNA was extracted from cells using commrtial KIT (Yekta Tajhiz Azma (YTA) Co., Iran Cat No. YT2551). After determination of RNA concentrations using spectrophotometric analysis, it was reverse transcribed to cDNA.

Real time analysis for all genes was performed in triplicate with a program having holding for 10 min at 95°C, 40 cycles of 95°C for 10 sec in the following, followed by annealing at 61°C for 20 sec, and 72°C for 20 sec along with a final extension of 72°C for 10 min(22).

Animal study

The atherosclerosis development was monitored in terms of foam cell formation, macrophage abundance and total intimal surface area of aorta in the rabbit model of atherosclerosis. In addition to rabbits with a normal diet as negative control, the plaques were induced by feeding rabbits with a high cholesterol diet (1%) within 8 weeks for other groups. Accordingly, 24 male New Zealand whites (Razi Institute) aged 6–8 weeks with a weight average of 8200 ± 50 gr were assigned to 4 groups including positive control, negative control, NP group (treated by PLGA nanoparticle) and NG group (treated by cell membrane-coated nanoparticle)(23). After plaque and induction, the treatment for NP and NG groups was started daily and twice a day for administration of NP and NG until 4 weeks, respectively, according to previous pharmacokinetic studies.

At the end of the study, the rabbits were intra-muscularly injected with ketamine/xylazine mixture (35 + 5mg/kg) to anesthetize them(24). The aorta, liver and kidney were removed from the scarified animals and immersed into training for future investigation. During treatment, the biochemical analysis in terms of glucose (BS), triglyceride (TG), cholesterol (Ch), SGPT (ALT), SGOT (AST) levels were monitored.

Histopathological study

The histologic investigation on samples was performed at Amapat Veterinary pathology special (Tehran, Iran) according to the protocol by Gomes et al with few modifications (ref). Briefly, portions of frozen tissue were cut and fixed. The section of 12µm were stained by hematoxylin and eosin before evaluation by the microscope.

Immunohistochemistry

Sections of aorta were typically stained in terms of pan-macrophage antigens CD14 (clone: MD85R, source: Rabbit, Isotype: IgG), CD68 (clone: KP1, source: Mouse Isotype: IgG1, kappa) and CD163 (source:
Rabbit, Isotype: IgG) as illustrated in the instructions (Diagnostic Biosystems, zytomed systems, Medaysis), respectively (25). The data was compared with non-immune antibodies (Isotype: IgG, source: Rabbit) according to instructions from MyBioSource. The population of stained macrophages in terms of CD14+, CD68 + and CD163 + biomarkers were assessed by a semi-quantitative estimation method (26).

**Statistical analysis**

The data was analyzed using SPSS (27.0.1), Excel (2022, x64) and GraphPad.Prism (v9.1.0.221.x64) software. The results were presented as the mean ± SD for triplicate measurements. For comparison of experimental groups, a P-value less than 0.5 was considered statistically significant regarding the difference in means which was assessed by a two-tailed t-test.

**Results**

RBC membrane-coated PLGA nanoparticles were prepared as mentioned and then characterized in terms of physiochemical and biological analyses.

**Preparation and characterization of PLGA nanoparticles and nanoghosts**

The PLGA nanoparticles were successfully prepared using solvent evaporation and then physiochemically characterized. The size measurement of the nanoghost by TEM showed a spherical particle with a size of about 125 nm having a membrane shell with a size of about 8.3 nm (Fig. 2A). The mean hydrodynamic size, polydispersity index and zeta potential of 185.4 nm, 0.152, -31 and those of 263 nm, 0.22 and -11 were obtained for the nanoparticle and nanoghost upon measurement by zeta sizer (Fig. 2B,C).

**Drug loading estimation and release study**

The loading capacity and encapsulation efficiency of Glyburide for the nanoghost were estimated to be 0.202% and 17%. The Glyburide release of 18.2% at 1h from the nanoghost was increased to 91.5% within 7th day. The dissolution profile and representative HPLC chromatogram of Glyburide were shown in Fig. 3A, B.

**Hemolysis assay**

The hemolysis assay for RBCs treated by the nanoghost in different concentrations showed hemolytic activity less than 4.5% within at a concentration range from about 0.33µg/mL up to 170µg/mL compared to positive control. Figure 3C shows the UV spectra of Hb release upon RBC hemolysis exposed to different concentrations of NG (27).

**Cytotoxicity and apoptosis assay**
An MTT assay of U937 cell line, exposed to different concentrations of nanoghost showed no significant toxicity. In other words, the cell viability percentages of more than 100% at 10µg/mL was decreased to about 83.2% at 500µg/ml (Fig. 4A). Moreover, no significant apoptosis was detected for the cell line exposed to NG and NP treatment as shown in Fig. 3B. Accordingly, there is no significant difference for number of alive cells between untreated and NG treated cells. However, a 5.6% decrease in the live cell population was observed for NP-treated cells.

**Estimation of anti-inflammatory mRNA levels**

An RT-PCR assay was directed to determine the mRNA levels in LPS-primed monocytes which were exposed to drug-loaded NP/NG in comparison to positive/negative controls. According to the results shown in Fig. 5, the mRNA levels of anti-inflammatory genes, including NLRP3, IL1-β, IL-18, Caspase-1, Caspase-8 and Caspase-9 were decreased to about 4.9, 19.16, 22.4, 98.81, 138.46 and 18.16 folds in LPS-primed monocytes-treated NG compared to positive control treatment (**p-value 0.004). The extent of decrease for the same mRNA in the cells exposed to NP was 2.77, 3.3, 5.16, 78.34, 122.23 and 133.89 folds compared to positive control treatment, respectively (**p-value 0.001).

**Anti-inflammatory evaluation of Glyburide-loaded NG/NP in an induced atherosclerotic rabbit model**

To determine the safe doses of NP/NG and their administration intervals, the systemic toxicity experiment and pharmacokinetic analysis were conducted according to a previously published article.

Rabbits were monitored by biochemical analysis of blood in TG, Cho, AST, ALT and Glu levels. While no alteration in ALT, AST and Glu levels was detected during feeding with a cholesterol enriched diet, TG and cholesterol amounts were decreased after treatment with NG and NP, to about 98% and 96%, respectively (Fig.S1).

After creating an atherosclerosis model and four-week treatment with NP and NG, the aorta of rabbits was isolated and immunohistochemical analysis was performed to investigate the levels of CD14, CD68 and CD163 biomarkers using 3, 3'-diaminobenzidine (DAB) labeled antibodies. In addition to the aorta, the liver and kidneys were also isolated and examined for hematoxylin and eosin (H&E) staining. As shown in Fig. 5, the CD14 + cell population in the intima area of aorta was decreased 1.62 and 1.3 folds for groups exposed to NG and NP in comparison with positive control (****P-value < 0.0001), respectively. Moreover, the differences for comparison of NG/NP treatments with normal control were statistically significant (****P-value < 0.0001). In this regard, the anti-inflammatory potential of NG was about 1.24 fold greater than NP (**P-value < 0.001) (Fig. 6.A and B).

Furthermore, NG and NP caused the 1.72 and 1.59 folds increase in CD163:CD68 ratio, in comparison with the positive group (**P-value < 0.002, Fig. 6C and D). This difference was also significant not only between NG/NP and normal group (****P-value < 0.0001) but also between NG and NP, which was 1.07 folds greater for NG treatment. (****P-value < 0.0001; Fig. 6.C and D).
H&D staining of sections taken from tunica intima/media for all groups was presented in Fig. 7A and Fig. 7B to estimate the extent of surface area and foam cells. In this regard, the estimation of total surface area in tunica intima taken for NG and NP groups showed a decrease of about 13.24 and 5.93 folds, compared to the positive group (**p-value < 0.0001; Fig. 7.C). Additionally, the potential of decreasing in the area was 2.23 folds for the NG than NP group, respectively (**p-value < 0.0001).

The total foam cell measurement for all groups was also facilitated by semi-quantitative analysis on H&D staining, where the total foam cell percentages were 45.6%, 21.6% and 38.7% for the positive, NG and NP group (Fig. 7D). In comparison with positive control, the potential of NG and NP in declining total foam cells was 2.1 and 1.17 folds (**p-value = 0.001, *p-value < 0.011), respectively (Fig. 7.D). The comparison between NP and NG treatment in decreasing total foam cell percentage was also significant (1.79 fold greater for NG treatment, ****p-value < 0.0001).

Finally, according to H&D staining for liver (Fig. S2A) and kidney (Fig. S2B) in all groups, the pathological issues which were detected in sections of liver and kidney in the positive group were reduced or disappeared upon NG and NP treatment like negative control. It should be noted that these pathological signs in positive control were hyperplasia, necrosis, and interim hepatitis. The order of potential in reducing the pathological effect and inflammation for NG was stronger than NP in all assessments.

**Discussion**

Atherosclerosis management by traditional medications has displayed disadvantages of nonspecific distribution, adverse effects and sometimes short circulation time for drugs. To remove these obstacles to atherosclerosis therapy, a biomimetic approach can be considered as a promising candidate with desired and safe properties. In this regard, the platform of cell membrane-coated nanoparticles, nanoghost, has recently been interested by scientists (28). Furthermore, since inflammation plays an essential role in the development of the disease, targeting the inflammatory pathways with the help of nanoghost can be considered an effective strategy for preventing the progression of the disease.

Considering the role of RBC membrane in plaque pathogenesis towards the site of inflammation, we decided to design a RBC membrane-based nanoghost. The expressed biomarker, CD47, on the RBC membrane surface also prevents phagocytosis by macrophage, giving a signal of “Don’t eat me”(29). In addition, to treat inflammation aspects of atherosclerosis, a sulfonylurea derivative drug, glyburide, was selected to load into the nanoghost, regarding its anti-inflammammaosome property(30).

To improve the solubility of the hydrophobic drug and its release pattern, the drug was encapsulated into PLGA nanoparticles. NG was fabricated using a mini-extrusion method to cover RBC membrane on PLGA membrane in which surface coating was confirmed by TEM and membrane sidedness assay. Furthermore, these NGs showed a larger size and a more positive zeta potential than NP due to surface coating.
The results of the dissolution profile also showed the adequate release of Glyburide from NG. Before each clinical study, a hemocompatibility assessment for nanomaterials was considered essential. In this regard, hemolysis assay in different concentrations of the nanoghost showed excellent hemolytic compatibility. Furthermore, estimation of cell viability percentage, as another criterion for biocompatibility, indicated no significant toxicity for NG concentration of less than 250 microgram/mL. The attenuation in expression levels of an anti-inflammatory gene using PCR assay exhibited anti-inflammatory effects of Glyburide in LPS-primed cells. Furthermore, the decrease in NLRP3 levels indicated the anti-inflammasome property of Glyburide as a sulfonyl urea derivative.

The anti-inflammatory results were further confirmed by in vivo study through immunohistological and pathological assays.

According to pathological results obtained from plaque sections, NG treatment of animals not only decreased foam cell abundance in tunica intima, but also decreased CD14-positive cell population compared to the positive control group, which confirmed the anti-inflammatory effects of the drug in the NG platform. Additionally, the increased ratio of CD163 macrophages to CD68 positive upon NG treatment ones compared to the positive control can show polarization of macrophages from M1 to M2 (31).

Our results were confirmed by the results obtained from rapamycin therapy for cholesterol-induced atherosclerosis lesions in rabbits (32).

According to our observation, the total surface area of plaque in tunica intima was significantly decreased for the NG treated group than the positive groups.

The extent of anti-inflammatory effects for NPs appeared slightly less than those for NG effects.

The anti-inflammatory effects had a straight relationship with decrease in TG and Cholesterol amounts after NG treatment.

In general, by taking advantage of the Glyburide in the NG-based platform including, hemo/biocompatibility, anti-inflammatory effects, M2/M1 polarization, decrease in plaque foam cells/surface area, Glyburide-loaded NG can be considered as a potential nanotherapeutic for the treatment of atherosclerosis and even other diseases have inflammatory manifestations.

**Conclusion**

In summary, we developed a nanoghost consisting of PLGA nanoparticles in a core compartment which was coated by RBC membrane for the treatment of inflammation aspects of atherosclerosis. The results confirmed the appropriate features in size and zeta potential, and drug loading. Besides bio/hemocompatibility, the inflammation indicators including foam cell numbers, total surface area and CD14 + macrophage were decreased by nanoghost treatment in the atherosclerosis model rabbits. This results together with the potential of nanoghosts in M1 to M2 polarization offers this platform as a
potential candidate for the control of inflammation-based complications caused by atherosclerosis and the management of other inflammatory diseases.

**Declarations**

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**Data availability**

Some datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study is included in this published article and its supplementary information files.

**Ethics statement**

All experimental procedures described in the present experiment were approved by Research Ethics Committee of Tehran University of Medical Sciences (Approval ID: IR.TUMS.TIPS.REC.1401.141)

**References**


Supplementary Information

Supplementary Information not available with this version.

Figures
Figure 1

Fabrication and evaluation of the red blood cell-based biomimetic platform (Glyburide-loaded nanoghost) for inflammation-based therapy of atherosclerosis.
Figure 2

Representative TEM micrograph of NG (A); hydrodynamic size of NP (B) and NG (C) measured by zeta sizer
Figure 3

(A) The dissolution profile of Glyburide release from the RBC-nanoghost; each point is an average of the triplicate measurement. (B) The representative HPLC chromatogram of Glyburide for drug release profile. (C) Uv-visible spectra of supernatant solutions in hemolysis assay for RBC treated by different concentrations of NG; The below photograph indicates the visual hemolysis of experimental samples in comparison with positive and negative controls.
Figure 4

(A) MTT assay of U937 cell line treated by nanoghost in the concentration range of 10-500 µg/ml. Error bar shows deviation of triplicate measurement (****P-value <0.0001). (B) Apoptosis assay by flow-cytometry through staining with annexin V-FITC/propidium iodide (PI); (a) Untreated cell, (b) unstained, (c) NP treated and (d) NG treated cells.
Figure 5

Expression levels of NLRP3, IL1-β, IL-18, Caspase-1, Caspase-8 and Caspase-9 for LPS-primed monocytes exposed to nanoghosts (NG) and nanoparticles (NP) compared to untreated cells. ** shows P-value<0.001. The data are reported as mean ±SD (n=3).
Figure 6

Representative immuno-histochemical staining for estimation of cell populations regarding (A) CD14+; (B) CD163+; (C) CD68+ for the aorta sections in the rabbit groups of negative control, NP treated, NG treated and positive control. Black arrows show the location of foam cells. Semi-quantitative estimation of (C) CD14+ cell percentage and (D) CD163:CD68 ratio. Data represent mean ± SD (n=3).
Figure 7

The representative hematoxylin-eosin staining for the aorta sections (A,B) in the rabbit groups of positive control (a), NG (b), NP (c) treated and normal control (d). Estimation of surface area (µm²) in intimal area of plaques (C) using the Optika software (x64, 4.11.18081.20201205. The estimation of the total foam cell percentage (D) for NG/NP treated groups, compared to the normal and the positive group (P value less than 0.05 indicates the data is statistically significant). Data represent mean ± SD (n=3).