



Semaglutide alleviates inflammation-Induced endothelial progenitor cells injury by inhibiting MiR-155 expression in macrophage exosomes

Xiaoyu Pan ^{a,b}, Lin Yang ^b, Shuqi Wang ^{a,b}, Yanhui Liu ^b, Lin Yue ^c, Shuchun Chen ^{a,b,*}

^a Department of Internal Medicine, Hebei Medical University, Shijiazhuang, China

^b Department of Endocrinology, Hebei General Hospital, Shijiazhuang, China

^c Department of Endocrinology, The Third Hospital of Shijiazhuang, Shijiazhuang, China



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ABSTRACT

The low-grade inflammatory state in obesity can damage vascular endothelial cells and lead to several cardiovascular diseases. Macrophage exosomes improve glucose tolerance and insulin sensitivity in obese mice, and yet it is unclear how it relates to endothelial cell injury. Firstly, lipopolysaccharide (LPS)-induced macrophage exosomes were co-cultured with endothelial progenitor cells (EPCs) to examine the function of EPCs and the level of inflammatory factors. Secondly, macrophages were transfected with MicroRNA-155 (miR-155) miR-155 mimics and inhibitors, and their secreted exosomes were co-cultured with EPCs to detect EPCs function and inflammatory factor levels. Then, EPCs were transfected with miR-155 mimics and inhibitors to clarify the effect of miR-155 on EPCs function and inflammatory factors. Finally, macrophages were intervened using semaglutide, and their secreted exosomes were co-cultured with EPCs to test EPCs function, inflammatory factor levels and macrophages miR-155 expression. LPS-induced macrophage exosomes reduced the cellular activity, migratory capacity and tube-forming ability of EPCs and rendered EPCs in an inflammatory state. LPS-induced macrophage exosomes significantly upregulated miR-155 expression. miR-155 high expression exacerbated the pro-inflammatory nature of macrophage exosomes and inhibited the cell viability of EPCs. In contrast, inhibition of miR-155 expression showed the opposite result, suppressing inflammation and increasing the cell viability of EPCs. Semaglutide improved the cell viability of EPCs and also inhibited the expression of inflammatory factors in EPCs as well as miR-155 in exosomes. Semaglutide improves the function and inflammatory status of EPCs may via inhibition of LPS-induced macrophage expression of miR-155 in exosomes.

1. Introduction

Obesity is a chronic metabolic condition that is frequently accompanied by several secondary pathological changes, such as insulin resistance, endothelial damage, and atherosclerosis [1,2]. Obesity's continuous chronic inflammatory state impairs intravascular homeostasis [3], as evidenced by raised pro-inflammatory factors interleukin-1beta (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and decreased anti-inflammatory factor interleukin-10 (IL-10) [4]. Endothelial cells are significant drivers of vascular function, and endothelial injury is the basis of many cardiovascular illnesses; consequently, it is clinically relevant to investigate the mechanisms and modulation of endothelial injury in obesity. When activated by physiological or pathological conditions, endothelial

progenitor cells (EPCs), also known as angioblast, can be moved from bone marrow to peripheral circulation to take part in the repair of damaged arteries. EPCs are crucial for maintaining the homeostasis of endothelial function. In order to better understand how chronic inflammation affects obesity and the mechanisms that contribute to it, this study was carried out.

Lipopolysaccharides (LPS) are a distinctive element of gram-negative bacteria's cell walls that help to preserve the integrity of the outer cell membrane. It can set off a chain reaction of immunological stimulation, toxic pathophysiological responses, and endotoxin release, which can result in infectious shock and peripheral vascular failure. Cellular investigations have verified that LPS may induce macrophage polarization and elicit inflammatory responses, and numerous research have demonstrated that it can be used to construct animal models of inflammatory reactions [5–8]. Recent research has demonstrated that

* Corresponding author at: Department of Internal Medicine, Hebei Medical University, Shijiazhuang, China.

E-mail address: chenshuc2014@163.com (S. Chen).

Table 1
Sequences of qRT-PCR primers.

Inflammatory Factor Gene	Primer Sequence (5'-3')
IL-1 β	F: ATCAGCACCTCTCAAGCAG R: AGTCCACATTCAAGCACAGG
IL-6	F: CCAGAGCTGTGCAGATGAGT R: GTGCCCATGCTACATTGCC
TNF- α	F: CCTGCTGCACCTTGGAGTGA R: ACAACATGGGCTACAGGCCIT
ICAM-1	F: AGCACTCAAGGGGAGGTCA R: GGGATAGGTTCAAGGGAGGC
VCAM-1	F: AGTAAGGCAGGCTGTAA A AGAA R: GTAGACCCCTCGCTGGAAACA
GAPDH	F: TCTCCTCTGACTTCACAGCGACA R: GGTGGTCCAGGGCTTACTCCTT

Abbreviation: IL-1 β , interleukin-1beta. IL-6, interleukin-6. TNF- α , tumor necrosis factor-alpha. ICAM-1, intercellular adhesion molecule-1. VCAM-1, vascular cell adhesion molecule-1. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

eating a high-fat diet over an extended period of time raises blood levels of LPS produced by gram-negative bacteria in the gut, and that these elevated levels of LPS in vivo are linked to low-grade chronic inflammation [9]. Low-grade systemic and adipose tissue inflammation are characteristics of obesity. As a result, LPS-induced macrophages were selected for this investigation to examine how the inflammatory state affects macrophage and EPCs function.

Exosomes are vesicles released by different cell types that range in size from 40 to 100 nm and include a lot of proteins, miRNAs, and lncRNAs. They play a key role in intercellular communication and control a number of physiological and pathological processes. Injecting exosomes released by M2-type macrophages from lean mouse adipose tissue into obese mice, for instance, increased glucose tolerance and insulin sensitivity in a model of obesity and insulin resistance [10]. Exosomes lacking miRNA 690, however, prevent the improvement of insulin sensitivity [10]. As a multifunctional miRNA, microRNA-155 (miR-155) has been shown to control the onset of several disorders [11]. Both studies have demonstrated that miR-155 is involved in the control of many blood vessels. For instance, miR-155 overexpression inhibits the ability of human umbilical vein endothelial cells to proliferate and migrate, while miR-155 silencing encourages the expression of inflammatory factors and apoptosis in these cells [12–14]. Further research is required to clarify the role and mechanism of miR-155 in the damage obesity-induced EPCs cause. MiR-155 may also be a possible new target in endocrine and metabolic illnesses as it has recently been

demonstrated that miR-155 in exosomes produced from adipose tissue macrophages in obese mice is implicated in the control of insulin resistance [15].

A brand-new medication for the treatment of diabetes mellitus called semaglutide works by targeting the glucagon-like peptide-1 receptor (GLP-1R). Semaglutide has been shown in numerous studies to have a weight loss effect and cardiovascular protection independent of its hypoglycemic effect. These effects are related to its inhibition of inflammatory oxidative stress, improvement of abnormal lipid metabolism, and alleviation of endothelial dysfunction in addition to a reduction in LPS-induced acute inflammation [16–18]. Although stimulation of the inflammatory state of macrophages occurs in conjunction with obesity, which is a chronic inflammatory disease, it is unknown if this activation of macrophages secretes exosomes that are responsible for the obesity-induced damage to EPCs. With the use of an LPS-induced macrophage model, this study will also examine the ameliorative effects of semaglutide on the harm that obesity-induced EPCs cause, and it will also investigate if the process is connected to the fact that macrophage exosomes carry miR-155 incredibly well.

2. Methods

2.1. Cell culture

Murine EPCs were provided by iCell Bioscience Inc, Shanghai (Item No. RAT-iCELL-0095a) and cultured in iCell primary EPCs medium (Item No. PriMed-iCell-013). Mouse macrophages RAW264.7, purchased from the Cell Center of the Chinese Academy of Medical Sciences (Beijing, China), were cultured in suspension passaged in normal DMEM medium containing 10% fetal bovine serum (FBS).

2.2. Study subgroups

The Raw264.7 macrophage cell line was divided into six groups: Control, 1 μ g/mL LPS group (LPS), Control + miR-155 mimic, Control + miR-155 inhibitor, LPS + miR-155 mimic, and LPS + miR-155 inhibitor; exosomes from each of the six macrophage groups were isolated after 24 h and incubated with EPCs co-cultured. The EPCs were divided into two groups: miR-155 mimics group and miR-155 inhibitors group. To investigate the effect of semaglutide, the RAW264.7 macrophage cell line was divided into three groups: Control group, LPS group, and LPS + 100 nmol/L semaglutide group. Exosomes from three groups of macrophages were isolated after 24 h and co-cultured with EPCs separately.

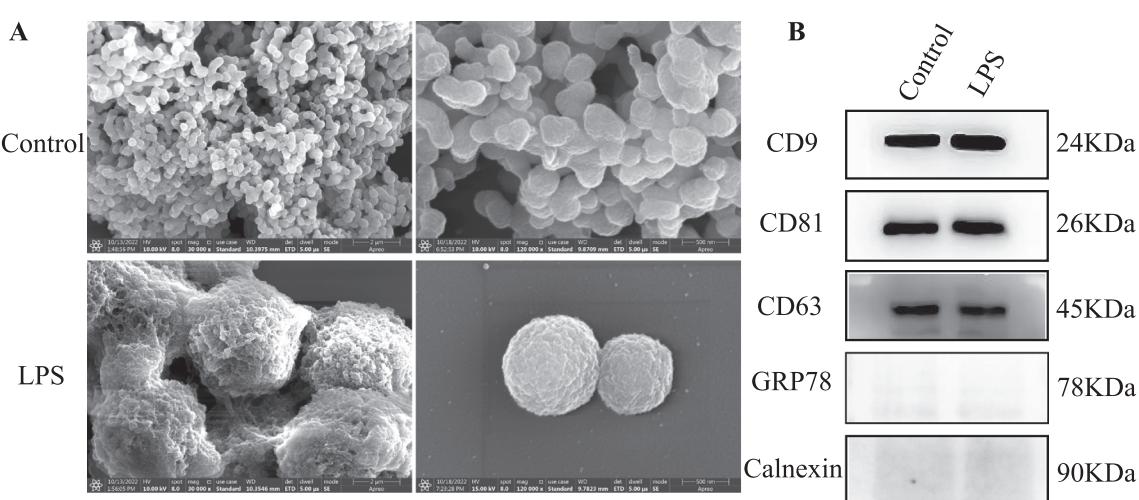


Fig. 1. Identification of LPS-induced macrophage exosomes. (A) Representative electron micrographs of exosomes. (B) Identification of exosome marker proteins. The experiments were performed at least three independent times.

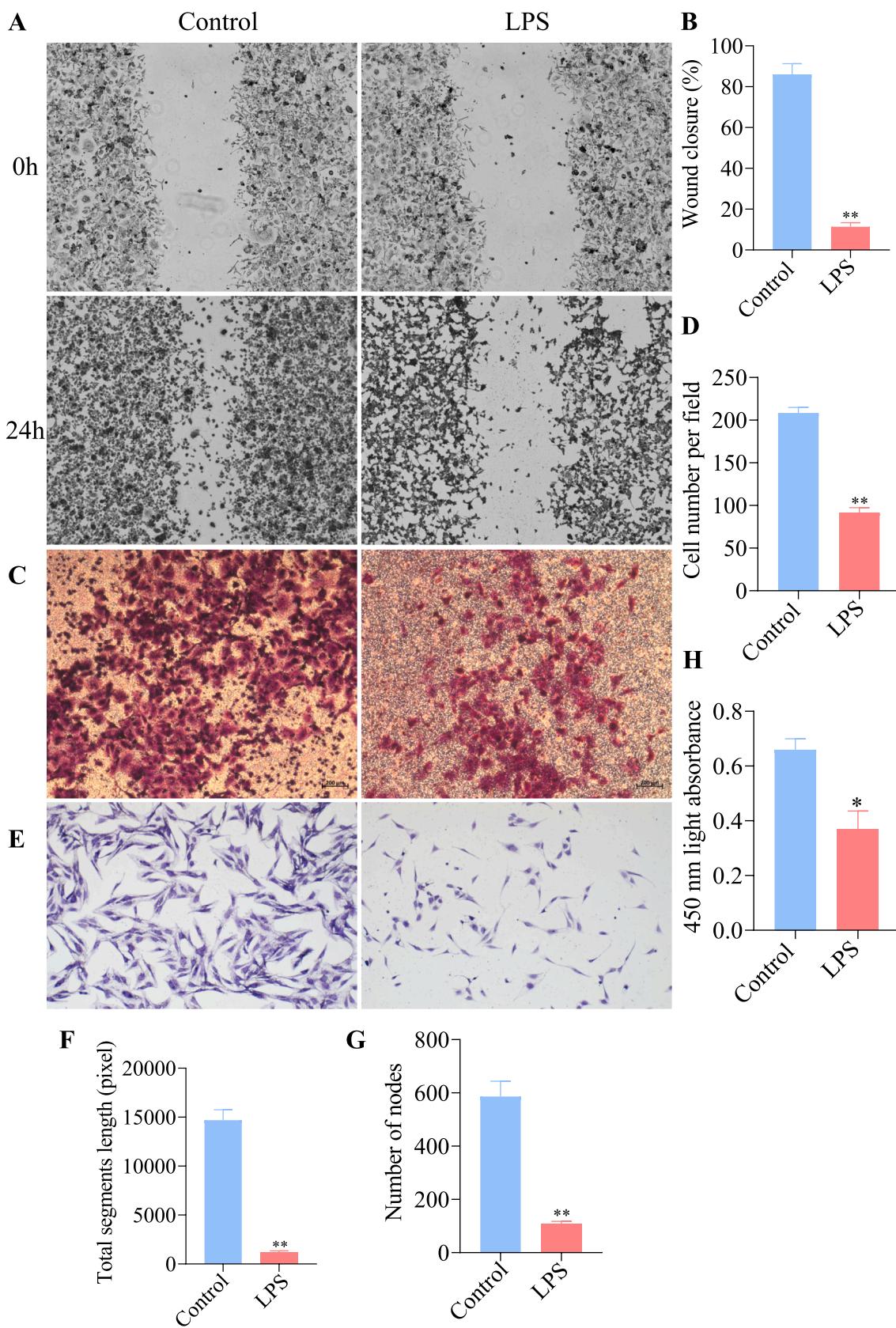


Fig. 2. Effect of LPS-induced macrophage exosomes on EPCs. (A and B) Scratch experiment (200 \times). (C and D) The transwell experiment (200 \times). (E-G) Tube formation experiments (200 \times). (H) Cell viability assay. *P < 0.05, **P < 0.01. The experiments were performed at least three independent times. Data were expressed as means \pm SEMs and analyzed by independent samples t-test.

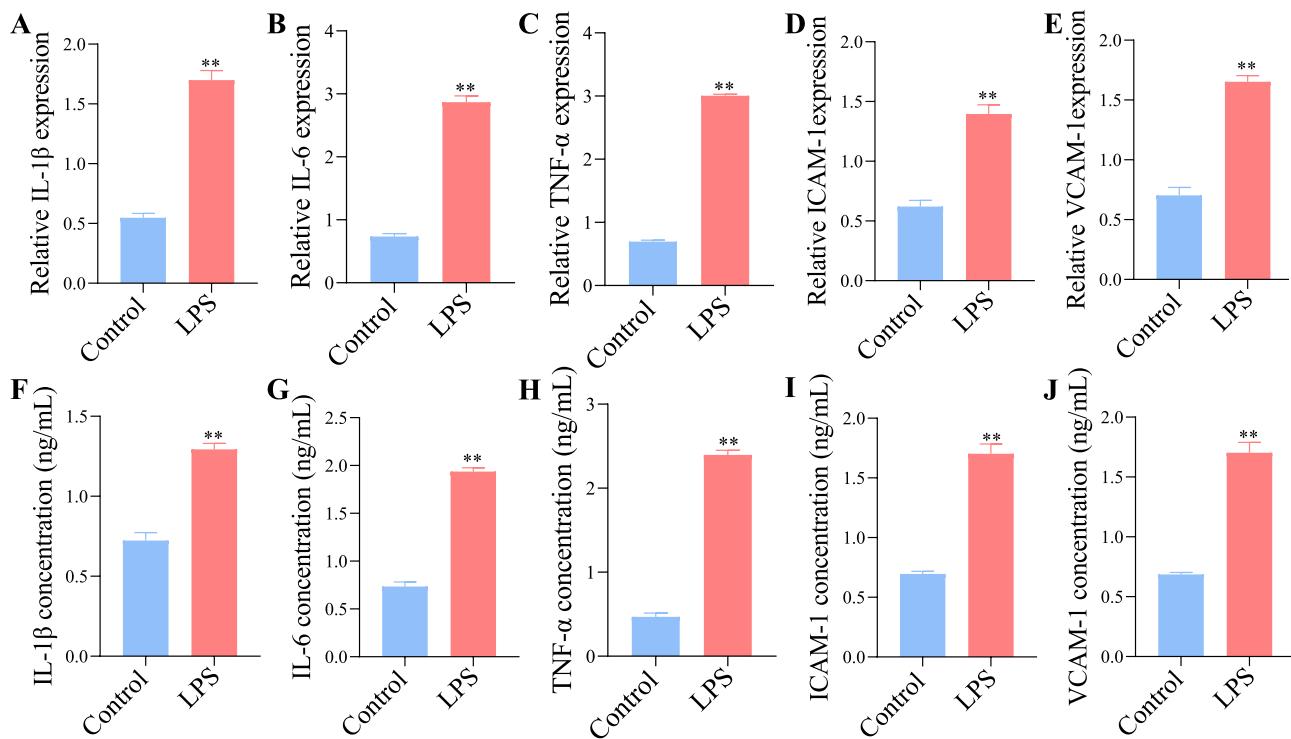


Fig. 3. LPS-induced macrophage exosomes expose EPCs to inflammatory states. (A-E) The expression of IL1- β , IL6, TNF- α , ICAM-1 and VCAM-1 was detected by RT-PCR. (F-J) The expression of IL1- β , TNF- α , IL6, ICAM-1 and VCAM-1 was detected by ELISA. **P < 0.01. The experiments were performed at least three independent times. Data were expressed as means \pm SEMs and analyzed by independent samples t-test.

2.3. Exosome isolation

The exosomes were extracted using the High Efficiency Exosome Precipitation Kit (Item No. EI-027) from invent Biotechnologies, INC (Beijing, China). The steps are briefly described as follows: collect 50 mL of culture medium, centrifuge at 2000 \times g for 10 min to collect the supernatant, centrifuge at 10,000 \times g for 30 min to collect the supernatant, filter once with 0.22 μ m filter membrane, centrifuge at 100,000 \times g for 30 min after precipitation of exosome precipitation reagent, resuspend with phosphate buffer solution (PBS) and wash once, centrifuge at 100,000 \times g for 70 min and finally resuspended with PBS.

2.4. Exosome Identification

Take 50 μ L of 2% paraformaldehyde solution and mix it with 20 μ L of exosome suspension. Add 5 μ L of the mixture onto the copper mesh and adsorb it in a dry environment for 20 min. Transfer the mesh to a PBS droplet for cleaning with forceps, and then transfer it to a 1% glutaraldehyde droplet. The copper mesh was then washed in distilled water and the washing was repeated 8 times. The copper mesh was transferred to a 50 μ L droplet of hydrogen peroxide acetate (pH 7.0) negatively stained, 50 μ L of methylcellulose droplet. The excess liquid on the side of the copper mesh was gently aspirated with filter paper and dried in air for 10 min. Observed and photographed under transmission electron microscopy with an accelerating voltage of 120KV. Western Blot detection of exosome marker proteins (CD9/CD63/CD81), as well as endoplasmic reticulum markers, Calnexin and anti-78kD glucose regulatory protein (GRP78) were used to identify the isolated particles as exosomes.

2.5. Cell viability detection

After cell counting, EPCs (100 μ L, 2 \times 10⁴ cells/well) with the same cell suspension volume were inoculated into 96-well culture plates and

grown on the wall for 12 h. The cells were replaced with MV2 cell basal medium and starved for 12 h. Different groups of exosomes (concentration 30 μ g/ml) were added to the EPCs and incubated for 24 h. After that, 10 μ L CCK-8 solution was added to each well for 4 h. The optical density values were measured in an enzyme-labeled instrument at a wavelength of 450 nm.

2.6. Wound healing test

A 6-well plate was inoculated with EPCs (2 \times 10⁴ cells/well), and after growing into a monolayer of cells, it was replaced with MV2 cell basal medium starved for 12 h. Cell debris was washed away by drawing a straight line along a straightedge with a 10ul white gun tip and incubated with medium containing 1% FBS for 24 h. After washing the cells, they were observed under an inverted microscope and photographed.

2.7. Transwell assay for migration

Migration activity was detected using 24-well Transwell plates (6.5 μ m wells) (Costar/Corning, Lowell, MA, USA). A total of 100 μ L, 1 \times 10⁴ EPCs were inoculated in the upper chamber of the Transwell, which was later replaced with MV2 basal medium without FBS. A total of 100 μ L of exosomes isolated from different treatment groups were added separately and treated for 24 h. After 500 μ L of MV2 complete medium was added to the lower chamber and the culture was continued for 6 h. The cells were fixed in 4% paraformaldehyde solution and stained with 0.05% crystal violet. Remove the cells above the strainer and rinse with PBS. The cells in the upper chamber were gently swabbed away, and the migration of each group of cells was observed in an orthomosaic microscope, and three random fields were selected to count the cells migrating into the lower chamber.

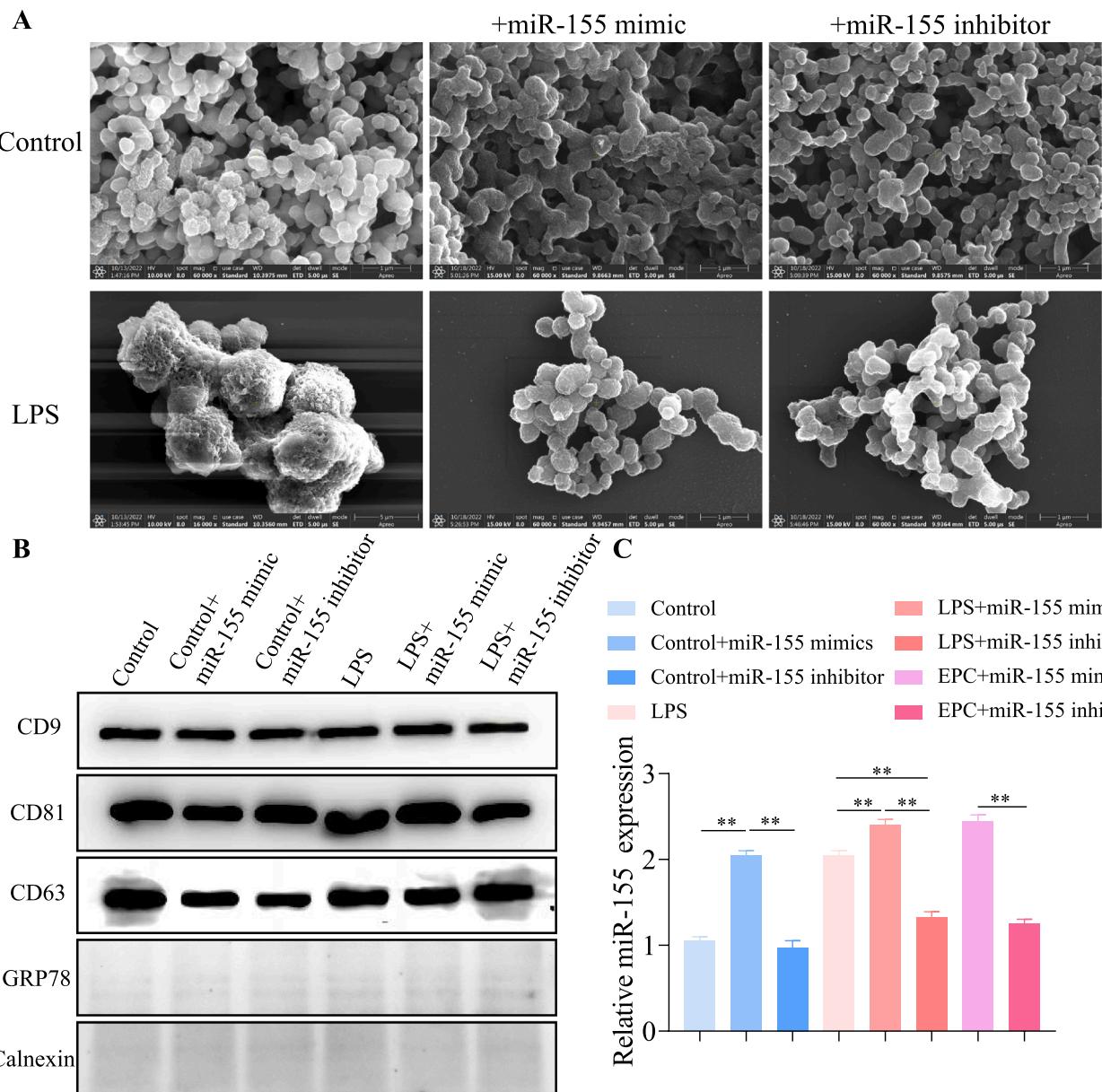


Fig. 4. MiR-155 transfection and exosome identification. (A) Representative electron micrographs of exosomes. (B) Identification of exosome marker proteins. (C) MiR-155 transfection results. **P < 0.01. The experiments were performed at least three independent times. Data are expressed as means ± SEMs and were tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test.

2.8. Tube formation

Matrigel (BD Biosciences, Bedford, MA, USA) was first dissolved at 4 °C and added at a concentration of 200 µL to each well of a 24-well plate and incubated at 37 °C for 15 min. A total of 500 µL of EPCs (containing 2×10^5 cells) were resuspended in MV2 serum-free medium and added to the wells after 24 h of treatment according to the different groups. After incubation at 37 °C for 6 h, the formation of EPCs tubes was examined by microscopy. The number of nodes and the total length of the tube formations were analyzed using ImageJ software.

2.9. Quantitative reverse transcription PCR (qRT-PCR)

The expression of inflammatory factors in EPCs after different treatments was analyzed. The primer sequences of IL-1β, IL-6, TNF-α, ICAM-1, VCAM-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown in Table 1. After extracting total RNA from EPCs

according to the instructions of TRIzol kit (Invitrogen), NanoDrop 2000 was used to determine the RNA concentration. RNA samples with 1 µg A260/A280 ratio between 1.8 and 2.0 were reverse transcribed, complementary DNA was synthesized using Prime Script RT kit (TaKaRa) and finally quantified by real-time PCR using SYBR Green Supermix kit (TaKaRa). Results were calculated using $2^{-\Delta\Delta CT}$ method.

2.10. Enzyme linked immunosorbent assay (ELISA)

IL-1β, IL-6, TNF-α, ICAM-1 and VCAM-1 kits were purchased from Nanjing Jiancheng Bioengineering Research Institute Co. Ltd. (Nanjing, China). The cell supernatant was taken and 100 µL of the solution containing inflammatory factors was added to the wells of the enzyme-labeled plate, and the concentration of the corresponding factors was measured by adding antibody, chromogenic substrate and termination solution respectively according to the instructions. The optical density value was read using an enzyme-labeled instrument.

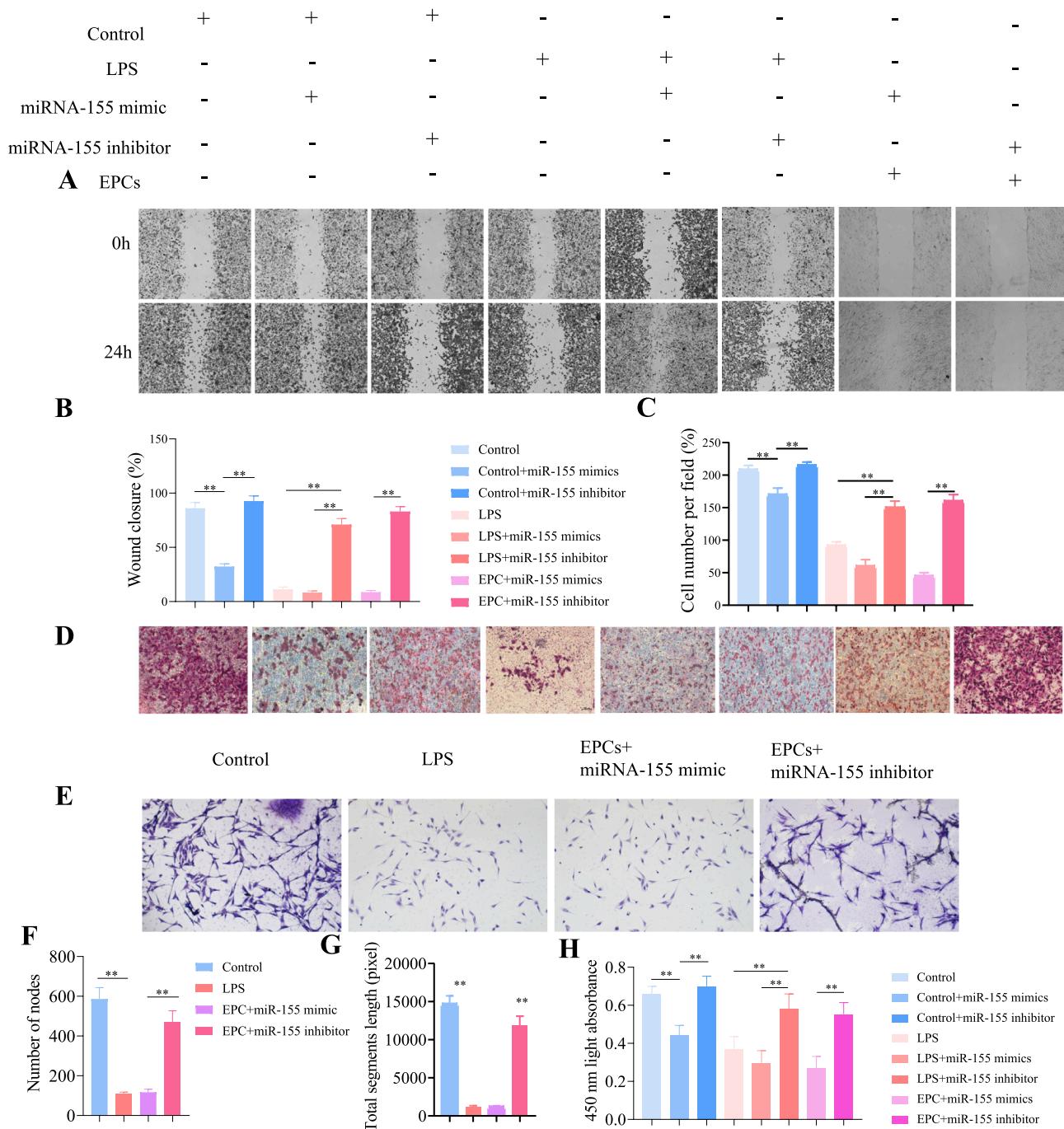


Fig. 5. Effect of miR-155 on EPCs in LPS-induced macrophage exosomes. (A and B) Scratch experiment (200×). (C and D) The transwell experiment (200×). (E-G) Tube formation experiments (200×). (H) Cell viability assay. **P < 0.01. The experiments were performed at least three independent times. Data are expressed as means ± SEMs and were tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test.

2.11. MiR-155 transfection

The miR-155 mimics and miR-155 inhibitors and their negative controls were transfected with the transfection reagent Lipofectamine™3000 (Invitrogen, CA, USA) in macrophages and EPCs. The efficiency of transfection and cell function assays were measured 48 h after transfection. Transfection sequences, miRNA-155 mimic sense: 5'-UUAAUGCUAUUGUGAUAGGGGU-3', anti-sense: 5'-CCCUAUCA-CAAUUAGCAUUAUUU-3'; miRNA mimics NC sense: 5'-UUCUCC-GAACGUGUCACGUTT-3', anti-sense, 5'-ACGUGACACGUUCUGGAGAA TT-3'; miRNA-155 inhibitor: 5'-ACCCCUCACAAUUAGCAUUA-3';

miRNA inhibitor NC, 5'-CAGUACUUUGUGUAGUACAA-3' (GenePharma Co., Ltd., Shanghai, China).

2.12. Statistical processing

All data were expressed as mean ± SD, and Graphpad 8.0 software was used for statistical analysis. Independent samples t-test was used for comparison between the two groups. Statistical analysis between multiple groups was tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test. P < 0.05 was defined as a statistically significant difference between groups.

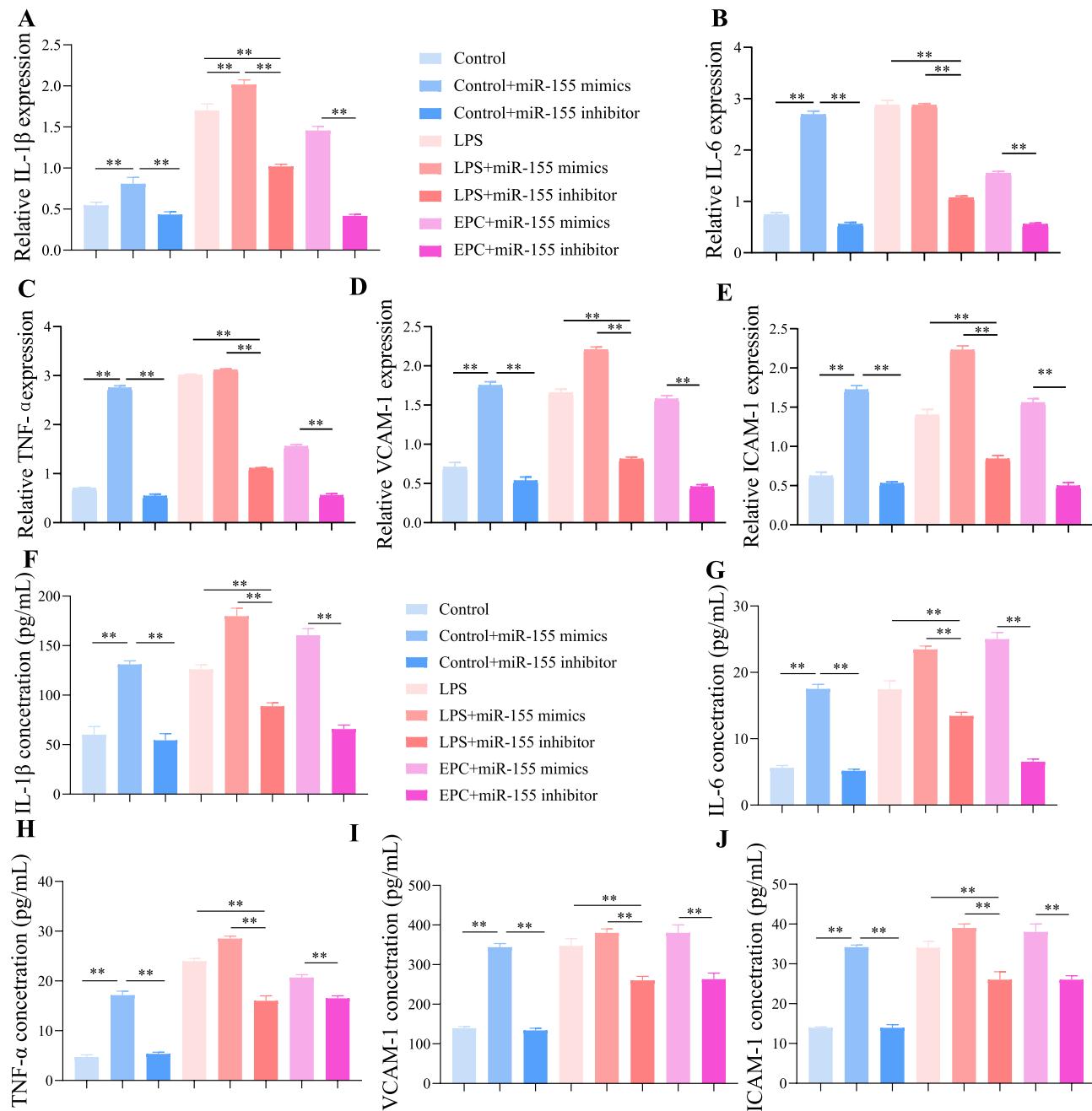


Fig. 6. Effect of miR-155 on inflammatory factors in EPCs. (A-E) The expression of IL1- β , IL6, TNF- α , ICAM-1 and VCAM-1 was detected by RT-PCR. (F-J) The expression of IL1- β , TNF- α , IL6, ICAM-1 and VCAM-1 was detected by ELISA. **P < 0.01. The experiments were performed at least three independent times. Data are expressed as means \pm SEMs and were tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test.

3. Results

3.1. Identification of LPS-induced macrophage exosomes

Morphological features of exosome suspensions can be observed from transmission electron microscopy. A large number of vesicle-like structures were observed in electron microscopy, with round or oval shapes and relatively uniform sizes, with diameters of about 40–100 nm. Electron microscopic observations did not reveal microvesicles, apoptotic vesicles, cellular debris and other subcellular organelles. The results of electron microscopy showed that the exosomes secreted by macrophages were not in clusters but relatively individually dispersed after LPS treatment, and the volume of exosomes increased. However, the total amount of exosomes secreted was not significantly reduced,

only the adhesion capacity on the electron microscopic copper grid was significantly decreased (Fig. 1A).

The results with Western blot showed high expression of CD9, CD63 and CD81, while Calnexin and GRP78 were not expressed, indicating that the vesicle-like structures extracted from the macrophage culture supernatant were exosomes (Fig. 1B).

3.2. Effect of LPS-induced macrophage exosomes on EPCs

Proliferation viability, migration and tube-forming ability of EPCs co-cultured with exosomes secreted by macrophages from both groups were examined. Scratch assay and transwell assay showed that LPS-treated exosomes were able to significantly reduce the migration ability of EPCs (P < 0.05) (Fig. 2A-D). Tube formation assay results showed

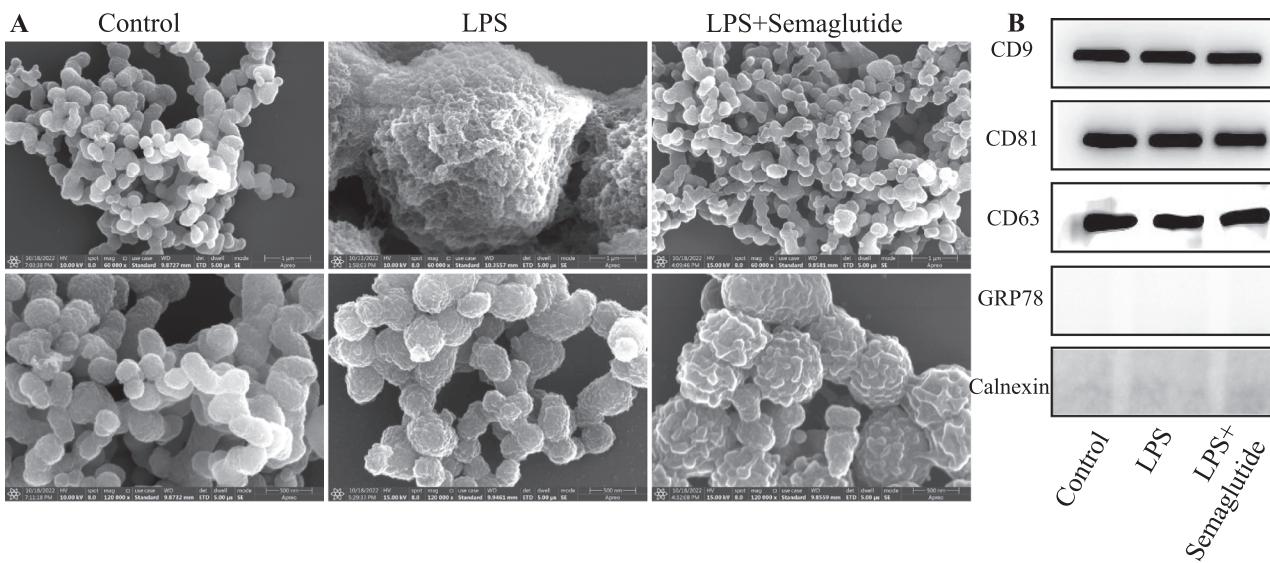


Fig. 7. Effect of semaglutide on macrophage exosomes. (A) Representative electron micrographs of exosomes. (B) Identification of exosome marker proteins. The experiments were performed at least three independent times.

that exosomes secreted by LPS-treated macrophages significantly reduced the tube-forming ability of EPCs. LPS-exosome resulted in a significantly lower number of nodes and total length of the tubular form of EPCs than controls ($P < 0.05$) (Fig. 2E–G). The cell counting kit-8 assay showed that LPS-induced exosomes secreted by macrophages reduced the cellular activity of EPCs, as evidenced by reduced 450 nm light absorption ($P < 0.05$) (Fig. 2H).

3.3. LPS-induced macrophage exosomes expose EPCs to inflammatory states

LPS-treated macrophages secreted exosomes that induced an inflammatory state in EPCs. RT-PCR confirmed that the expression of IL-1 β , TNF- α , IL-6, ICAM-1, and VCAM-1 were significantly elevated in the LPS-exosome treated group ($P < 0.01$) (Fig. 3A), and ELISA results also indicated that these inflammatory factors and chemokines were also significantly elevated in the cell supernatants ($P < 0.01$) (Fig. 3B).

3.4. MiR-155 transfection and exosome identification

To clarify whether miR-155 plays a role in the injury process of EPCs, we transfected macrophages and EPCs with miR-155. The electron microscopy results of isolated exosomes showed that LPS-treated macrophage exosomes were transfected with miR-155 or inhibited miR-155 with little change in their exosome morphology. However, compared to the exosomes secreted by macrophages without LPS treatment, their exosomes had decreased direct adhesion ability and slightly different particle size (Fig. 4A). In addition to electron microscopy, we examined exosomal biomarkers (CD9, CD63, CD81) and endoplasmic reticulum markers (Calnexin, GRP78) by Western blot, which confirmed high expression of exosomal markers and no expression of endoplasmic reticulum markers (Fig. 4B).

The content of miR-155 in macrophage exosomes was detected, and snRNA U6 was used as an internal reference. The results showed that LPS-induced macrophages resulted in a significant upregulation of miR-155 expression in exosomes. Normal macrophages, LPS-induced macrophages and EPCs showed corresponding up- and down-regulation of expression after transfection with miR-155 mimics and inhibitors, indicating that miR-155 mimics and miR-155 inhibitors successfully transfected macrophages and enhanced and decreased miR-155 content in exosomes accordingly. (Fig. 4C).

3.5. Effect of miR-155 on EPCs in LPS-induced macrophage exosomes

Post-transfection RT-PCR results showed that LPS stimulation of macrophages caused upregulation of miR-155 expression, and we further investigated the cellular activity of different treatments and cells after transfection with miR-155 mimics and inhibitors. Both scratch assay and transwell assay confirmed that overexpression of miR-155 inhibited the motility and migratory ability of EPCs and suppressed the ability of tubule formation. In contrast, inhibition of miR-155 expression promoted the motility and migratory ability of EPCs and enhanced their ability to form tubules (Fig. 5A–G). High miR-155 expression inhibited the cell viability of EPCs, whereas inhibition of miR-155 expression showed the opposite result, increasing the cell viability of EPCs (Fig. 5H). High expression of miR-155 exacerbated the pro-inflammatory nature of macrophage exosomes, whereas inhibition of miR-155 expression alleviated the inflammatory state (Fig. 6 A and B).

3.6. Effect of semaglutide on macrophage exosomes

From the results of electron microscopy, semaglutide did not have a significant effect on the 3D structure and appearance of exosomes secreted by macrophages (Fig. 7A). Western blot detection of exosomal and endoplasmic reticulum marker proteins secreted by macrophages treated with semaglutide showed high expression of CD9, CD63 and CD81, while Calnexin and GRP78 were not expressed (Fig. 7B).

3.7. Semaglutide protects EPCs by inhibiting macrophage exosome miR-155 expression

To determine the protective effect of semaglutide on EPCs, 100 nmol/L semaglutide intervened in LPS-induced macrophages. The proliferation viability, migration and tube-forming ability of EPCs co-cultured with macrophage exosomes were detected. The results showed that semaglutide had a more significant improvement ($P < 0.01$) on the migratory (Fig. 8A–D), tube-forming (Fig. 8E–G) and proliferative (Fig. 8H) abilities of EPCs. The results of miR-155 assay suggested that semaglutide significantly inhibited miR-155 in macrophage exosomes ($P < 0.01$) (Fig. 8I). In addition, Elisa and qRT-PCR results showed that semaglutide significantly reduced the expression of IL-1 β , IL-6, TNF- α , ICAM-1 and VCAM-1 in EPCs and culture medium ($P < 0.01$) (Fig. 9A and B). Therefore, we speculate that the protective effect of semaglutide

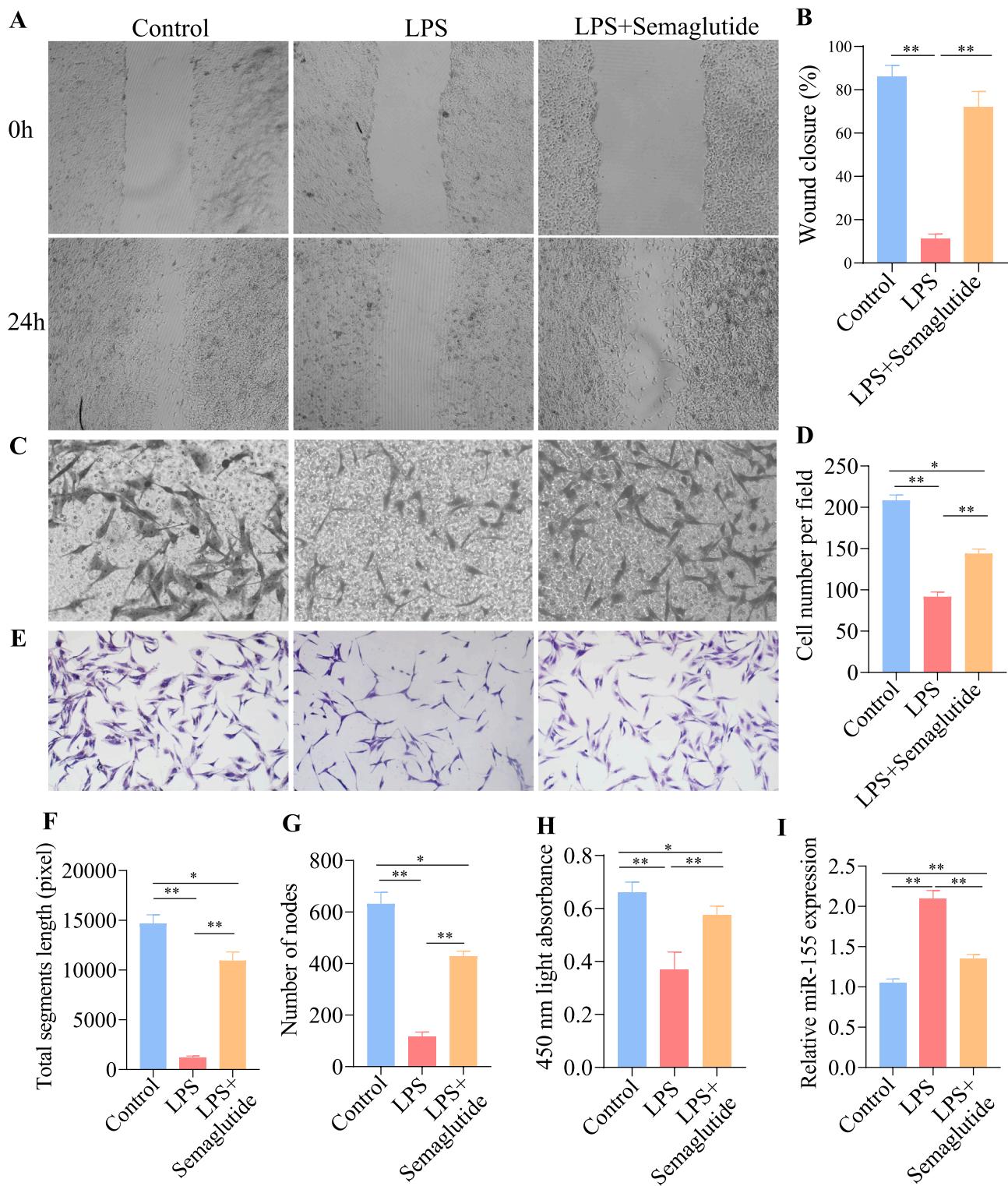


Fig. 8. The effects of semaglutide on the function of EPCs. (A and B) Scratch experiment (200 \times). (C and D) The transwell experiment (200 \times). (E-G) Tube formation experiments (200 \times). (H) Cell viability assay. (I) Effect of semaglutide on macrophage exosome miR-155 expression. *P < 0.05, **P < 0.01. The experiments were performed at least three independent times. Data are expressed as means \pm SEMs and were tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test.

on EPCs may act by inhibiting the expression of miR-155 in exosomes.

4. Discussion

The risk of dying from being overweight or obese is the fifth greatest in the world, and obesity can result in a number of issues that reduce

patients' quality of life and shorten their lifespan [19]. Investigating the processes of obesity in the emergence of associated disorders is crucial for this reason. Recent research has demonstrated that low-grade adipose tissue inflammation in obesity might impair EPCs function and result in microangiopathy [20]. The inflammatory status of adipose tissue in obesity is partially linked to macrophage infiltration [21].

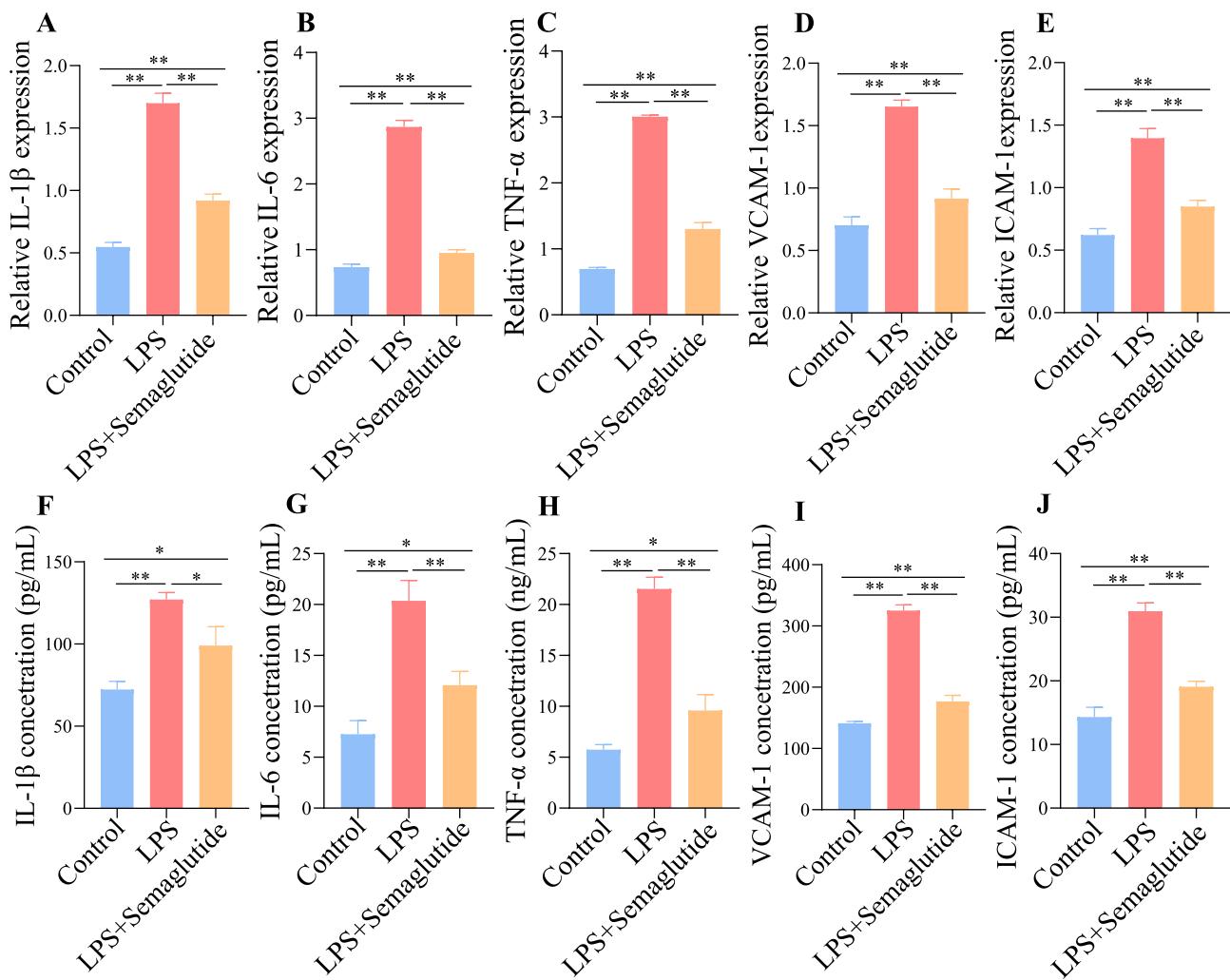


Fig. 9. Effect of semaglutide on inflammatory factors in EPCs. (A-E) The expression of IL1- β , IL6, TNF- α , ICAM-1 and VCAM-1 was detected by RT-PCR. (F-J) The expression of IL1- β , TNF- α , IL6, ICAM-1 and VCAM-1 was detected by ELISA. *P < 0.05, **P < 0.01. The experiments were performed at least three independent times. Data are expressed as means \pm SEMs and were tested by one-way analysis of variance (ANOVA) using Tukey's post hoc test.

Moreover, exosomes produced by macrophages have the ability to merge with EPCs, promote the production of inflammatory factors in these cells, and affect their ability to migrate, proliferate, and create lumens [22]. Exosomes released by macrophages can therefore affect EPCs. Earlier research has demonstrated that LPS can activate TLR-4 in adipocytes and macrophages, resulting in inflammation in adipose tissue [23]. In order to examine the impact of macrophage exosomes on the functionality of EPCs, we co-cultured macrophage released exosomes with these cells in this work after using LPS to generate macrophages. According to the research, LPS-induced macrophage exosomes can increase the expression of inflammatory markers in EPCs and decrease their viability, migratory capacity, and tube-forming capacity. Our finding shows that inflammation and EPCs dysfunction are tightly related to LPS-induced macrophage exosomes. According to earlier research, LPS-induced macrophage exosomes may control microglia polarization to provide neuroprotection following ischemic stroke [24]. Moreover, alterations in the metabolism of adipose tissue were linked with changes in the expression of many MiRNAs in LPS-induced macrophage exosomes [25].

Uncertainty exists regarding the mechanism underlying the functional impairment of EPCs caused by LPS-induced macrophage exosomes. Exosomes can transfer miR-155 to endothelial cells, cause endothelial damage, and encourage atherosclerosis, according to earlier research [26]. Furthermore, miR-155 causes endothelium-dependent

vasodilatory dysfunction and lowers NO production [27]. It has been discovered that miR-155 can bind to patched-1 (PTCH1) and suppress its expression when blood sugar levels are high. SHH can encourage the proliferation of EPCs through the PI3K/Akt pathway by binding to PTCH1, starting signaling, and enabling migration because PTCH1 is a receptor of the SHH signaling pathway. In contrast, large levels of miR-155 can inhibit SHH signaling and cause PTCH1 expression to be downregulated, which in turn impairs EPCs function and speeds up apoptosis [28]. We investigated whether miR-155 in macrophage exosomes was related to EPCs dysfunction based on the aforementioned findings. Despite the fact that LPS had no discernible impact on the quantity of exosomes released by macrophages, we discovered that it did raise the expression of miR-155 in exosomes. The function of EPCs was considerably diminished by LPS-induced macrophage exosomes, according to results of co-culturing them with macrophages. We carried out miR-155 transfection to determine whether miR-155 in exosomes is a crucial component in the malfunctioning of EPCs. Similar changes were seen in the expression of miR-155 in exosomes released by macrophages treated with miR-155 mimics and inhibitors. High miR-155 expression was linked to lower vitality, migratory capacity, and tube-forming ability of EPCs, while miR-155 expression inhibition improved LPS-induced dysfunction of EPCs, according to co-culture of the transfected macrophage exosomes with EPCs. According to the aforementioned findings, miR-155 in macrophage exosomes appears to

be crucial for the functional control of EPCs. As LPS-induced macrophage exocytosis places EPCs in an inflammatory state, which also appears to be correlated with lower viability, migratory capability, and tube-forming potential of EPCs, it is now known that inflammation affects the function of EPCs and vascular endothelial cells. Similar to how high miR-155 expression enhanced EPCs' inflammatory state, miR-155 expression inhibition alleviated this condition, demonstrating the anti-inflammatory capabilities of miR-155.

Semaglutide is a common diabetic medication that not only lowers blood sugar but also has a better impact on the body's cardiovascular and metabolic systems. Further research is needed to fully understand and confirm the potential and mechanism of this medication. In serum and cardiac tissues of obese mice, semaglutide was reported to reduce levels of inflammation and oxidative stress, although the precise mechanism is unknown [29,30]. By reducing the expression of Serpinh1 and Pcolce in fibroblasts, semaglutide delayed cardiac fibrosis, as revealed by the single-cell transcriptome of non-cardiomyocytes from fat mice [31]. Semaglutide reduces the negative effects of obesity on the heart and modifies the expression of numerous proteins involved in lipid metabolism in cardiac tissue [30]. Also, research on aortic proteomics has revealed that semaglutide alters the expression of a number of cellular matrix-related proteins and lessens the damage to the aorta caused by obesity [32]. Yet, it is unclear exactly how semaglutide affects vascular endothelial cells. In this work, semaglutide's functional effects on EPCs were examined under *in vitro* circumstances. The findings indicated that semaglutide might reduce the inflammatory state and enhance EPCs function. It was discovered that semaglutide drastically decreased the expression of miR-155 in exosomes by looking for its presence there. According to the aforementioned findings, miR-155 in macrophage exosomes can influence the inflammatory condition and functionality of EPCs, and semaglutide appears to protect EPCs by preventing miR-155 production in exosomes.

However, there are several shortcomings in our study. Firstly, due to the technical difficulties in isolating and storing exosomes, the function of exosomes was inevitably affected to some extent during the experiment, so *in vivo* experiments are needed to verify the results of this study. Secondly, the uptake of exosomes by EPCs was not performed in this study, and further studies are needed to clarify the specific molecular mechanism. Finally, the optimal intervention concentration for the protective effect of semaglutide on EPCs is unclear, and more subgroups are needed to determine it.

5. Conclusions

After LPS stimulation, macrophage exosomes impair the proliferative viability, migration, and tube-forming capacity of EPCs, leaving them in an inflammatory state. The function and degree of inflammation of EPCs appear to be controlled by miR-155, which is found in macrophage exosomes. By inhibiting miR-155 expression in macrophage exosomes, semaglutide may safeguard the function of EPCs.

Ethics approval.

All experimental procedures and animal management procedures were approved by the Ethics Committee of Hebei General Hospital (Approval Number: 2022-85; Date of approval: May 11, 2022).

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CRediT authorship contribution statement

Xiaoyu Pan: Conceptualization, Investigation, Formal analysis, Project administration, Writing – original draft. **Lin Yang:** Conceptualization, Project administration, Writing – review & editing, Funding acquisition. **Shuqi Wang:** Resources, Investigation. **Yanhui Liu:**

Investigation. **Lin Yue:** Resources. **Shuchun Chen:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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Not applicable.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2023.110196>.

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