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Research Article

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Mitochondria derived from hESC-MSCs alleviate inflammatory response in HGFs

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Abstract: Periodontitis is a common oral disease caused by bacteria coupled with an excessive host immune response. Stem cell therapy can be a promising treatment strategy for periodontitis, but the relevant mechanism is complicated. This study aimed to explore the therapeutic potential of mitochondria from human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) for the treatment of periodontitis. The gingival tissues of periodontitis patients are characterized by abnormal mitochondrial structure. Human gingival fibroblasts (HGFs) were exposed to 5 μg/mL of lipopolysaccharide (LPS) for 24 h to establish a cell injury model. Either when treated with hESC-MSCs or with mitochondria derived from hESC-MSCs, HGF showed a reduced expression of inflammatory genes, increased ATP levels, decreased ROS production, and enhanced mitochondrial function compared to the control. The average efficiency of isolated mitochondrial transfer by hESC-MSCs was determined to be 8.93%. Besides, a therapy of local mitochondrial injection in mice with LPS-induced periodontitis showed a reduction in inflammatory gene expression, as well as an increase in both mitochondrial number and aspect ratio in gingival tissues. In conclusion, our results indicate that mitochondria derived from hESC-MSCs can reduce the inflammatory response and improve mitochondrial function in HGFs, showcasing that the transfer of mitochondria between hESC-MSCs and HGFs serves as a potential mechanism underlying the stem cell therapeutic effect.

Key words: hESC-MSCs; Mitochondrial transfer; Inflammatory response; Mitochondrial dysfunction; Periodontal disease

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1 Introduction

Periodontitis is an inflammatory disease that can damage the soft and hard tissues supporting the teeth. Human gingival fibroblasts (HGFs), crucial to the connective tissue of gums, play an important role in the defense against pathogenic bacteria and restoring tissue health. However, excessive host immune responses can lead to harmful effects, such as tissue destruction and an imbalance in the oxidation-reduction system (Chapple and Matthews, 2007; Liu et al., 2017).

The mechanisms by which mesenchymal stem cells (MSCs) mitigate periodontitis are multifaceted. A particularly noteworthy element is mitochondrial transfer, as mitochondrial dysfunction plays a critical role in the pathogenesis and treatment of periodontitis (Jiang et al., 2023) (Huang et al., 2020). Recent research has shown that mitochondrial dysfunction is present in gingival tissues (Sun et al., 2017), and abnormal mitochondrial structures have been observed in gingival tissues and HGFs derived from patients with periodontitis

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(Liu et al., 2021b; Liu et al., 2022). The above studies underscore the significant role of mitochondria in the pathogenesis of periodontitis and signify mitochondrial dysfunction as a promising therapeutic target.

The transfer of healthy mitochondria from MSCs to injured target cells is an ongoing research topic (Liu et al., 2021a). While mitochondrial transfer has shown promise in alleviating severe inflammatory symptoms in conditions like asthma, stroke and myocardial infarction (Paliwal et al., 2018), limited research has been conducted on its role in periodontitis.

Human embryonic stem cell-derived MSCs (hESC-MSCs) are a subtype of MSCs derived from pluripotent stem cells (PSC-MSCs). Compared to MSCs derived from somatic tissues (st-MSCs), PSC-MSCs have achieved superior therapeutic effects through mechanisms such as mitochondrial transfer, paracrine signaling, and exosomes (Seo and Jeon, 2022).

This study analyzed the inflammatory response and mitochondrial function after treatment with hESC-MSCs and their derived mitochondria. The objective was to explore the ability of hESC-MSCs to reduce the inflammatory response and mitochondrial dysfunction in HGFs and to elucidate the role of mitochondria in the rescue process. Our findings contribute to the understanding of mitochondrial transfer between stem cells and damaged somatic cells, offering valuable insights into a novel approach for stem cell therapy in periodontitis.

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2 Results

2.1 Abnormal mitochondrial structure and cell injury were observed in the gingival tissues of patients with chronic periodontitis

Firstly, to determine the changes of mitochondria, we examined the mitochondria in chronic periodontitis (CP) and healthy controls (HC) by transmission electron microscopy (TEM). We found that the mitochondria in CP had abnormal structures, with swelling due to membrane disruption and the disappearance of cristae. In comparison, mitochondria in HC were more oval in shape, with larger aspect ratio and clear cristae (Fig. 1a).

Next, we assessed cell injury in CP. The collagen fibers were loosely arranged and disordered, and there was a higher density of inflammatory cells compared to HC (Fig. 1b). To demonstrate cell injury, DNA breakage was detected by TUNEL staining, and more positive points were observed in the nuclei in CP (Fig. 1c).

We also detected the expression levels of certain genes and proteins related to the inflammatory process. The mRNA level of IL-6, NLRP3, and IL-1β in gingival tissues were higher in CP than HC (Fig. 1d), along with a higher protein expression of NLRP3, the stimulated form of Caspase-1, and IL-1β (Fig. 1e).

2.2 Mitochondrial dysfunction was detected in HGFs induced by LPS

To determine the concentration of LPS that induces inflammatory response and mitochondrial dysfunction, we initially exposed HGFs to various concentrations of LPS (0, 0.1, 0.5, 1, 5, and 10 μg/mL). We found that LPS raised the mRNA expression of inflammatory factors (IL-6, IL-1β) and NLRP3, particularly at 5 μg/mL (Fig. 2a). Our data also revealed that cell viability decreased when the LPS concentration exceeded 1 μg/mL (Fig. 2b).

When comparing the mitochondrial function of HGFs treated with or without 5 μg/mL LPS, we observed a lower fluorescence ratio of aggregates versus monomers in HGFs stimulated by LPS, indicating that LPS treatment at that concentration resulted in a reduced mitochondrial membrane potential (Figure 2c). LPS-stimulated HGFs generated more ROS than the control group (Figure 2d). Regarding mitochondrial respiration, LPS at 5 μg/mL significantly diminished both ATP production and basal respiration in HGFs (Figure 2e).

In summary, we demonstrated that LPS at 5 μg/mL induced inflammatory response and mitochondrial dysfunction in HGFs. Hence, this concentration was chosen for the subsequent experiments.

2.3 hESC-MSCs reduced inflammatory response in HGFs after mitochondrial transfer

As previously mentioned, we constructed a model of mitochondrial dysfunction using 5 μg/mL LPS treatment on HGFs, so as to explore the rescue capabilities of hESC-MSCs. Three techniques are available to treat injured HGFs with hESC-MSCs, such as indirect transwell co-culture (Group IC), direct co-culture (Group DC), and conditioned medium of hESC-MSCs culture (Group Sup) (Figure 3a).

Initially, we performed cell membrane staining for HGFs and mitochondrial staining for hESC-MSCs, followed by 24-h direct co-culture (Group DC). Notably, we observed mitochondria with green staining within the HGFs, which confirmed the transfer of mitochondria from hESC-MSCs to HGFs (Figure 3b). Then, we quantified the efficiency of mitochondrial transfer by hESC-MSCs using flow cytometry (Figure 3c). The average efficiency was determined to be 33.55%.

Next, to further evaluate the rescue effects of hESC-MSCs, we set up indirect co-cultures using transwells (Group IC) and a conditioned medium of hESC-MSCs cultures (Group SUP), and detected the supernatant of Group DC, IC and SUP. The results showed that Group DC had the lowest IL-6 concentration in the supernatant among these groups (Figure 3d). Both Group IC and Group SUP showed the ability to alleviate the inflammatory response in HGFs, with reduced mRNA expression levels of inflammatory markers (IL-6, IL-1β, TNF-α) and NLRP3 (Figure 3e).

A comparison between Group DC, IC, and SUP suggested that hESC-MSCs alleviated inflammation in various ways. Notably, Group DC exhibited a relatively better effect on reducing IL-6 was shown in. Meanwhile, Group IC showed better effects than Group SUP on reducing mRNA expression levels of IL-1β and NLRP3, indicating that the existence of hESC-MSCs made a difference and the transfer of mitochondria could be responsible. We then performed cell membrane staining for HGFs and mitochondrial staining for hESC-MSCs as previously described but in Group IC. Interestingly, we found mitochondria with green staining in the HGFs, indicating the transfer of mitochondria from hESC-MSCs to HGFs through transwell assay. To prevent the influence of the dyeing solution, we used the supernatant of the last wash buffer (Figure 3f).

2.4 Mitochondria derived from hESC-MSCs reduced inflammatory response and mitochondrial dysfunction in HGFs

In order to gather additional evidence regarding the functionality of the transferred mitochondria in HGFs, we conducted an experiment to isolate mitochondria from hESC-MSCs and co-culture them with HGFs (Group Mito). HGFs either indirectly cultured with hESC-MSCs or co-cultured with exogenous mitochondria showed elevated mitochondrial membrane potential (Figure 4a) and diminished ROS production (Figure 4b). The mitochondrial function of HGFs appeared to be a little stronger in indirect cultures than in co-cultures with mitochondria, while the difference was insignificant.

In addition, we found that isolated mitochondria significantly improved mitochondrial respiratory function in HGFs, as indicated by increases in basal respiration, ATP production, maximal respiration, and spare respiratory capacity (Figure 4c).

To substantiate the occurrence of mitochondrial transfer to HGFs, we performed staining followed by isolating the mitochondria from hESC-MSCs and co-culturing them for 24 h. Staining revealed the presence of green mitochondria within HGFs (Figure 4d). Additionally, we assessed the efficiency of mitochondrial transfer by flow cytometry (Figure 4e), with an average efficiency of 8.93%.

Moreover, we observed that HGFs co-cultured with isolated mitochondria displayed lower mRNA expression levels of inflammatory markers, including IL-6, TNF-α, IL-1β, and NLRP3 (Figure 4f). The protein expression levels of IL-1β, NLRP3, GSDMD, and activated Caspase-1 also decreased (Figure 4g).

2.5 Mitochondria derived from hESC-MSCs enhanced the recovery of experimental periodontitis in mice

We established an experimental periodontitis mouse model to evaluate the therapeutic effects of hESC-MSC-derived mitochondria (Figure 5a). After treatment with mitochondria, there were fewer injured cells in the gingival tissues (Figure 5b). We assessed the mRNA expression of inflammatory markers, including

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NLRP3, IL-1β, IL-6, and TNF-α. Intriguingly, the levels of NLRP3, IL-1β and TNF-α showed significant reductions after being treated with mitochondria, whereas the IL-6 levels increased (Figure 5c). The decreased expression of NLRP3, IL-1β, and TNF-α in the Group MITO indicated reduced inflammatory response and enhanced recovery.

In order to examine the potential changes in mitochondrial morphology within gingival tissues, we analyzed the mitochondrial substructures using TEM. In the Group CTRL, numerous swollen and enlarged vacuolar mitochondria were observed within cells. Conversely, mitochondria in cells from the Group MITO displayed an oval shape with a larger aspect ratio and well-defined cristae (Figure 5d). These observations provided evidence that mitochondrial health and structure had improved in the MITO group.

3 Discussion

In this study, we identified a phenomenon wherein mitochondria from hESC-MSCs can be transferred into HGFs and mitigate the inflammatory response, as well as ameliorate LPS-induced mitochondrial dysfunction in HGFs. Additionally, mitochondria derived from hESC-MSCs could effectively rescue injured HGFs and facilitate recovery in an experimental mouse model of periodontitis. These findings highlight the therapeutic potential of mitochondrial transfer in periodontal treatment.

Our observations also revealed that inflammatory response and mitochondrial dysfunction can be ameliorated by MSCs. This concurs with findings of earlier studies that documented the anti-inflammatory effects and modulation of mitochondrial function through MSC therapy (Paknejad et al., 2015; Rodríguez-Fuentes et al., 2021). For instance, MSC-derived exosomes have been reported to improve alveolar-capillary barrier properties by restoring mitochondrial function (Dutra Silva et al., 2021). Studies have also shown that MSCs and their exosomes can counteract cigarette smoke-induced mitochondrial dysfunction in mice (Maremanda et al., 2019; Li et al., 2021) and human umbilical cord-derived MSCs can mitigate age-associated skeletal muscle dysfunction by modulating mitochondrial damage in mice (Piao et al., 2022). Although these investigations were not conducted in the context of periodontal disease, they underscore the potential of MSCs to regulate mitochondrial function in a more general scope.

We observed isolated mitochondria from hESC-MSCs transferred to HGFs and detected enhanced mitochondrial function and reduced inflammatory response in damaged cells. These results align with earlier studies in that transferring healthy mitochondria into damaged cells can improve mitochondrial biosynthesis, aerobic respiration, and ATP production while reducing reactive stress (Levoux et al., 2021). Transferring healthy mitochondria from stem cells to supplant damaged ones in injured cells has emerged as a promising therapeutic strategy for periodontitis. Mitochondrial transfer has been observed in MSCs derived from various tissues, such as bone marrow, umbilical cord, and fat (Shanmughapriya et al., 2020). Some MSC-derived extracellular vesicles (MSC-EVs) contain mitochondria that can transfer to human small airway epithelial and pulmonary microvascular endothelial cells (Ren et al., 2020).

On the other hand, treatment with isolated mitochondria did not attain therapeutic advantages over indirect co-culture. This was reasonable because the compounds secreted by MSCs can also boost periodontal function (Chew et al., 2019; Regmi et al., 2019; Nakao et al., 2021). Therefore, mitochondrial transfer is considered to play a role in the hESC-MSCs rescue effect.

Tunneling nanotubes (TNTs), extracellular vesicles (EVs) and free mitochondrial capture are the most popular routes of intercellular mitochondrial transport (Borcherding and Brestoff, 2023). The mechanism of mitochondrial transfer in restoring injured cells remains unclear but it is always related to cell energy metabolism and mitochondrial quality control. Under pathological conditions, healthy mitochondria are transferred from donors into recipients to replace the damaged ones and thus improve cell respiration recovery, biogenesis, and decrease oxidative stress (Liu, et al., 2021a). Mitochondrial transfer contributes to the classic mitochondrial quality control mechanism, which includes mitophagy, mitochondrial fission/fusion, mitochondrial biogenesis,

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and proteome quality control. The natural transfer of mitochondria from MSCs and the artificial transplantation of exogenous mitochondria could also trigger mitophagy in recipient cells, resulting in cytoprotective effects, including an enhanced engraftment capability (Lin et al., 2024). In addition, transferred mitochondria can act as stimulants. It was reported that by transferring a small number of mitochondria, exogenous mtDNA can readily replace or integrate with endogenous mtDNA in the recipient cells (Liu et al., 2023). However, the mechanism of mitochondrial effect after transfer needs further exploration.

The rescue effect of healthy isolated mitochondria may relate to the inhibition of pyroptosis, with ROS overdose being the key signaling event of pyroptosis. Typical pyroptosis occurs through inflammasomes and GSDMD pathways. LPS can induce NLRP3 and caspase-1, then stimulate GSDMD. The cleaved N-terminal domain anchors to the cell membrane, causing the leakage of cell contents, such as IL-1β (Broz et al., 2020). In this study, we tested the protein expression of NLRP3, GSDMD, Caspase-1, and IL-1β, and the results showed that mitochondria derived from hESC-MSCs decreased the expression of pyroptosis-related proteins in HGFs stimulated by LPS. The mice treated with mitochondria also presented a significant inhibition on the gene expression of NLRP3 and IL-1β. The mechanism between mitochondrial transfer and the pyroptosis of target cells is an interesting issue for further exploration.

Although no specific report has focused on mitochondrial transfer using hESC-MSCs, several studies have highlighted the benefits of using PSC-MSCs. For example, it has been found that induced pluripotent stem cell-derived MSCs (iPSC-MSCs) possess a higher capacity for mitochondrial transfer compared to bone marrow-derived MSCs (BM-MSCs), effectively mitigating mitochondrial damage induced by cigarette smoke (Li et al., 2014). This increased efficiency is attributed to the higher levels of intrinsic MIRO1, a mitochondrial Rho-GTPase that regulates intercellular mitochondrial movement, in iPSC-MSCs rather than BM-MSCs (Zhang et al., 2016).

However, we noted a higher mitochondrial transfer efficiency than that reported in other studies, where the percentage of transferred mitochondria averaged around 5% without additional stimulation (Anitua et al., 2013). This discrepancy could be attributed to the centrifugation process applied in this study, which may have enhanced the transfer efficiency (Caicedo et al., 2015). As we observed, the uptake of exogenous mitochondria increased proportionally to the quantity of mitochondria used, and the transfer rate could reach as high as 92.7 ± 5.9% (Kim et al., 2018).

This study has certain limitations. Firstly, we did not directly evaluate mitochondrial function in HGFs co-cultured with hESC-MSCs. The reason is that although a direct co-culture would offer a more comprehensive investigation of mitochondrial transfer, it could lead to reduced cell quantity and vitality. Secondly, it was difficult to explain the paradoxical changing trend of IL-6 expression when treating mice with isolated mitochondria. This observation revealed the complexity of mitochondrial therapy *in vivo*; it was reported that mitochondria transfer may trigger IL-6 classic signaling and generate anti-inflammatory functions (Xu et al., 2024). Additionally, our animal models of experimental periodontitis may not fully replicate the complexities of chronic periodontitis in humans. Despite these limitations, our findings offer initial evidence of the rescue effect of hESC-MSC-derived mitochondria.

4 Conclusions

In summary, our study demonstrated that hESC-MSCs can reduce the inflammatory response and improve mitochondrial function in HGFs via mitochondrial transfer. Moreover, mitochondria derived from hESC-MSCs maintained their ability to ameliorate damaged HGFs and inflamed gingival tissues. These results highlight the critical role of mitochondria in periodontitis and suggest the promising value of mitochondrial therapy for stem cell-based treatments. However, the regulatory mechanisms governing mitochondrial transfer and their influence on chronic periodontitis warrant further investigations.

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Materials and methods

The full material and methods are provided in the electronic supplement of this paper.

Data availability statement

The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments

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Author contributions

Bicong GAO: Contributed to conception and design, contributed to acquisition, analysis, and interpretation, and drafted and critically revised manuscript. Chenlu SHEN: Contributed to the acquisition, drafted, and critically revised manuscript. Kejia LV: Contributed to the acquisition, drafted, and critically revised manuscript. Xuehui LI: Contributed to analysis. Yongting ZHANG: Contributed to analysis. Fan SHI: Contributed to analysis. Hongyan DIAO: Contributed to conception and design, contributed to interpretation, drafted and critically revised manuscript. Hua YAO: Contributed to conception and design, contributed to interpretation, drafted and critically revised manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Bicong GAO, Chenlu SHEN, Kejia LV, Xuehui LI, Yongting ZHANG, Fan SHI, Hongyan DIAO, and Hua YAO declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the Research Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China) (No. IIT20221030A) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for which identifying information is included in this article. All experiments on animals were approved by the Animal Ethics Committee of The First Affiliated Hospital, College of Medicine, Zhejiang University (No. 2022-1601).

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Supplementary information

Table S1; Figs. S1-S3; Materials and methods

Figures and legends

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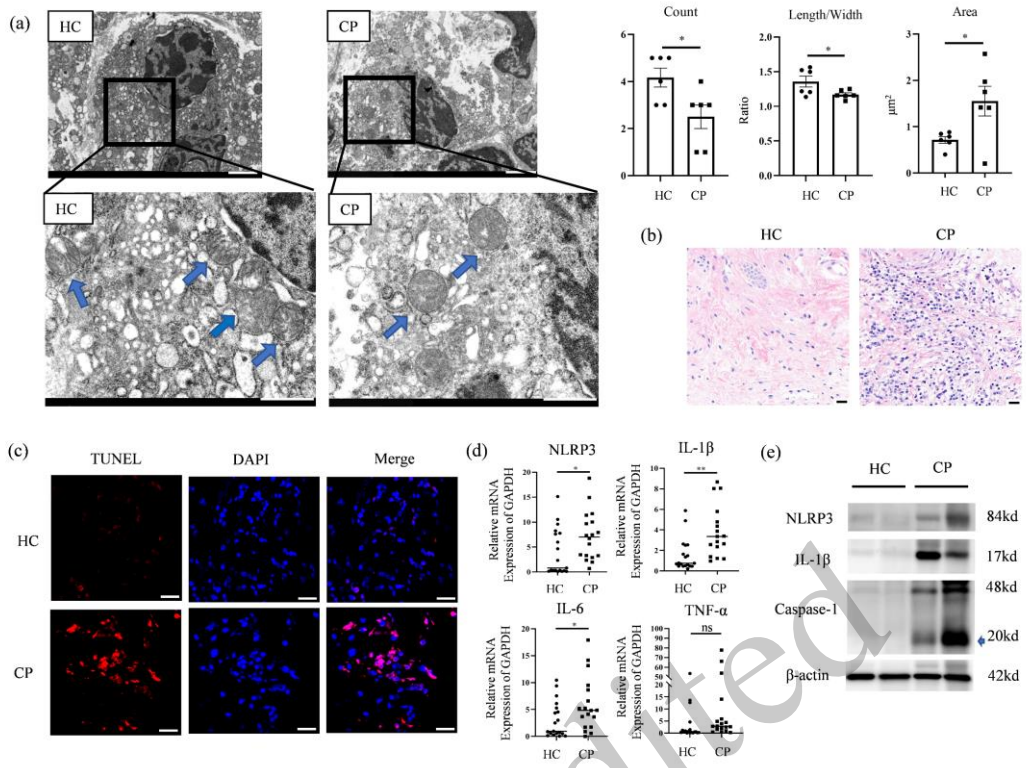

Figure 1 Abnormal mitochondrial structure and cell injury are observed in gingival tissues of patients with chronic periodontitis. (a) The substructure of mitochondria in gingival tissues was observed by TEM. The blue arrows indicate mitochondria. The mitochondria in HC are normal in size with a larger aspect ratio, and the double membrane structure and cristae are clear. The mitochondria from CP show abnormal structure including swelling, enlargement, and loss of cristae. Scale bar, 2 μm (upper) and 1 μm (lower). The mitochondrial count, length/width ratio, and area were calculated (n=6). (b) The structure of gingival tissues from donors with or without chronic periodontitis stained with HE. Scale bar, 20 μm. (c) Immunofluorescence assay of TUNEL. Scale bar, 20 μm. (d) Relative mRNA expression of NLRP3, IL-1β, IL-6, and TNF-α in gingival tissues in HC (n=17) and CP (n=19). (e) The protein expression levels of NLRP3, IL-1β, Caspase-1, and β-actin were measured by western blot. Data were presented as means ± SEM. Unpaired two-tailed Student's t-tests were conducted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: not significant. HE: hematoxylin and eosin; DAPI: 4',6-diamidino-2-phenylindole; TUNEL: TdT-mediated dUTP nick end labeling; HC: healthy control; CP: chronic periodontitis; TEM: transmission electron microscopy; NLRP3: NOD-like receptor family pyrin domain-containing 3; SEM: standard error of mean.

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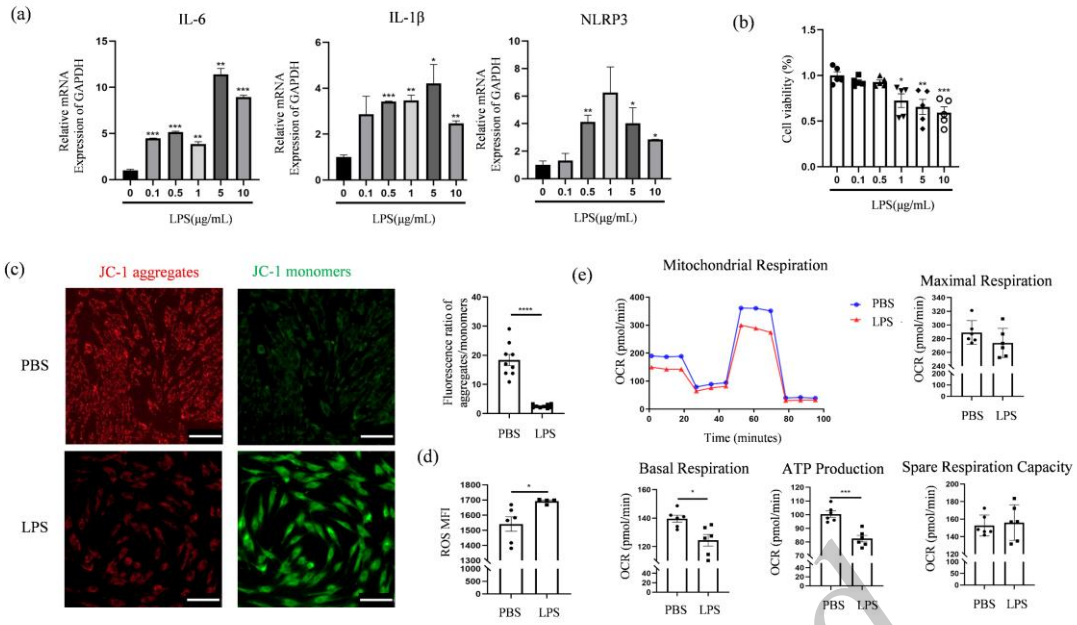

Figure 2 LPS induced the inflammatory response and mitochondrial dysfunction of HGFs. HGFs were treated with different concentrations (0, 0.1, 0.5, 1, 5, and 10 μg/mL) of LPS (a, b). (a) Relative mRNA expression of NLRP3, IL-6, IL-1β, and TNF-α. (b) Cell viability was determined by CCK8 assays. HGFs were treated with or without LPS of 5 μg/mL (c, d, e). (c) Representative fluorescence images of JC-1 aggregates (red) and monomers (green). The fluorescence ratio of aggregates to monomers was analyzed (n=9). (d) MFI of ROS were analyzed by flow cytometry (n=6). (e) Representative Seahorse Mito Stress assay. Calculated values for respiratory parameters: basal respiration, ATP production, maximal respiration, and spare respiratory capacity (n=6). Data were presented as means ± SEM; Unpaired two-tailed Student's t-tests were conducted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to Group PBS. LPS: lipopolysaccharide; PBS: phosphate buffered saline; ATP: adenosine triphosphate; ROS: reactive oxygen species; NLRP3: NOD-like receptor family pyrin domain-containing 3; MFI: median fluorescence intensity; SEM: standard error of mean.

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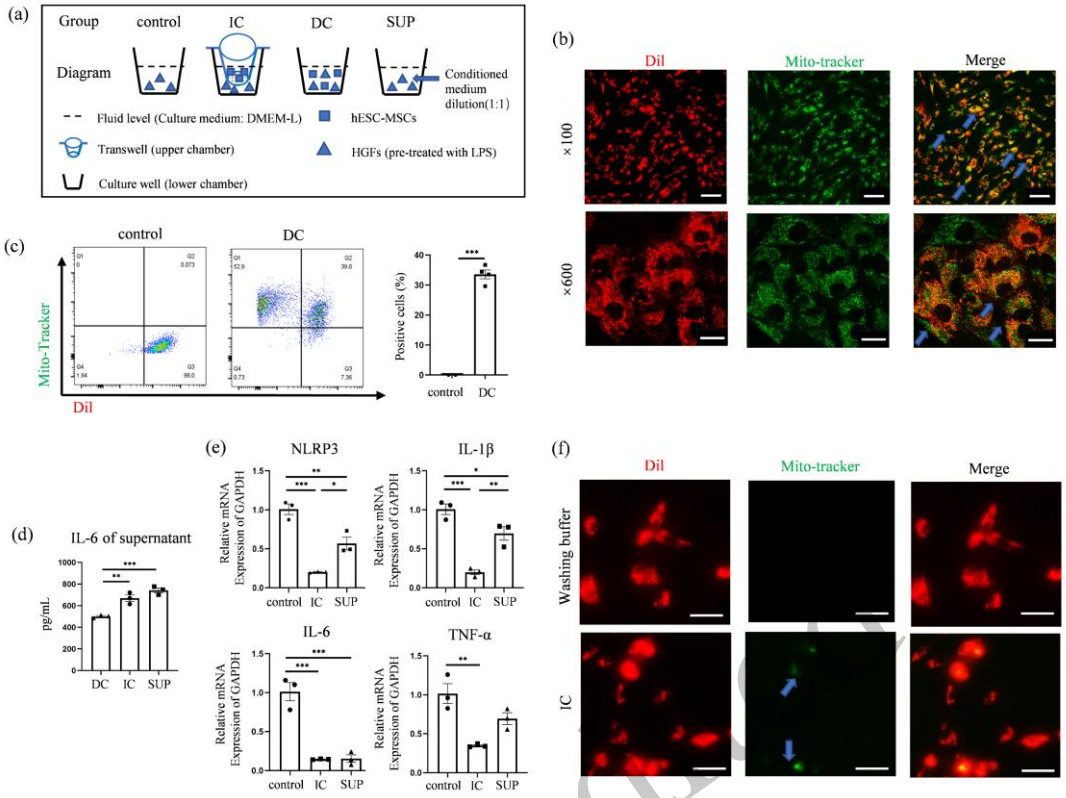

Figure 3 hESC-MSCs reduced the inflammatory response in HGFs through mitochondria. HGFs were pre-treated with LPS at 5 μg/mL. (a) Grouping diagram: HGFs were cultured alone (Group control), indirectly co-cultured with hESC-MSCs by transwells (Group IC), directly co-cultured with hESC-MSCs (Group DC), or cultured with conditioned medium supernatant of hESC-MSCs (Group Sup). (b) Confocal fluorescence image of Group DC. Mitochondria from hESC-MSCs were labeled with Mito-Tracker (green) and HGFs were stained with Dil (red). They were directly co-cultured for 24 h. The arrows show that the mitochondria derived from hESC-MSCs transferred to HGFs. Scale bar, 100 μm (upper) and 20 μm (lower). (c) Representative scatter chart of control and DC. The transfer subset (FITC+/PE+) cells were detected by flow cytometry (n=4). (d) IL-6 content of supernatant after 24 h of culture. (e) Relative mRNA expression of NLRP3, IL-1β, IL-6, and TNF-α. (f) Fluorescence image of Group IC. HGFs were cultured with the last time washing buffer during Mito-Tracker staining (upper). HGFs were cultured with hESC-MSCs labeled with Mito-Tracker by transwells (lower). The arrows show mitochondria from hESC-MSCs in HGFs. Scale bar, 100 μm. Data were presented as means ± SEM. Unpaired two-tailed Student's t-tests were conducted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to the group control. IC: indirectly co-cultured; DC: directly co-cultured; SUP: cultured with conditioned medium supernatant; NLRP3: NOD-like receptor family pyrin domain-containing 3; SEM: standard error of mean.

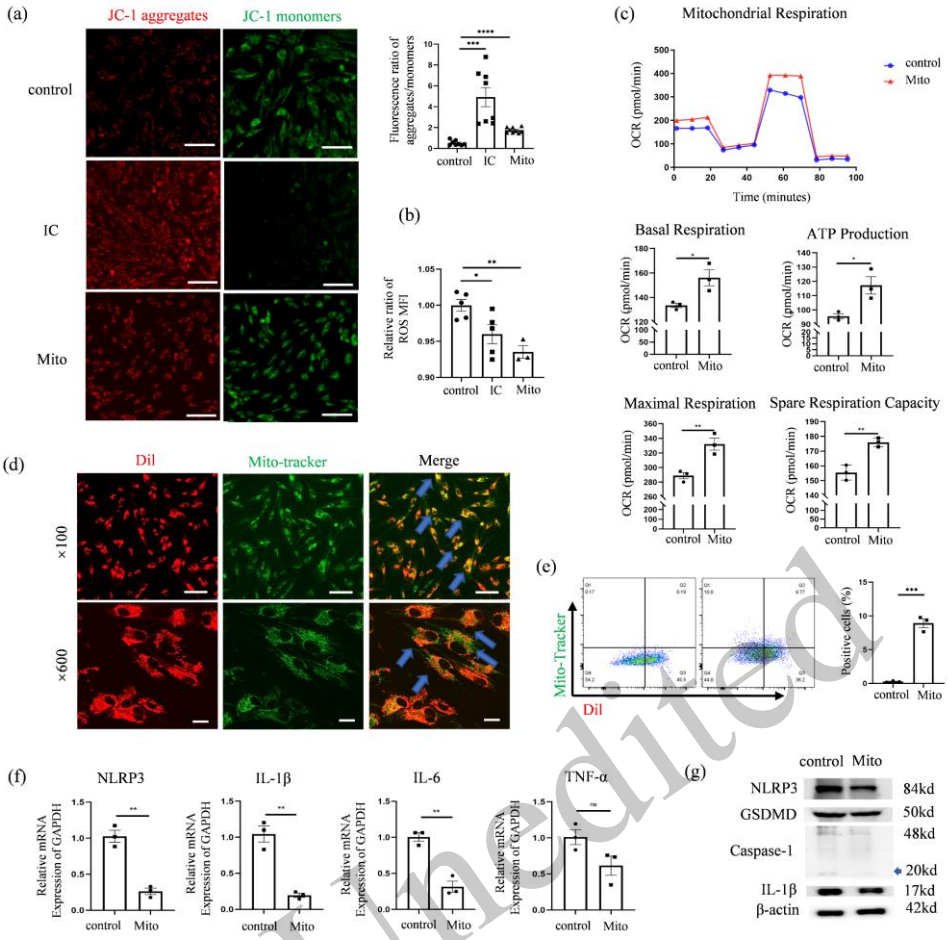

Figure 4 Mitochondria derived from hESC-MSCs reduced the inflammatory response and mitochondrial dysfunction in HGFs. HGFs were pretreated with LPS at 5 μg/mL. HGFs were cultured with mitochondria derived from hESC-MSCs (Group Mito), indirectly cultured with hESC-MSCs (Group IC), or cultured alone (Group control). (a) Representative fluorescence images of JC-1 aggregates (red) and monomers (green). The fluorescence ratio of aggregates to monomers was analyzed (n=8). (b) The MFI of ROS were analyzed by flow cytometry (n=3 or 5). (c) Representative Seahorse Mito Stress assay. Calculated values for respiratory parameters: basal respiration, ATP production, maximal respiration, and spare respiratory capacity (n=3). (d) Confocal fluorescence image of Group Mito. Mitochondria derived from hESC-MSCs were labeled with Mito-Tracker (green) and HGFs were stained with Dil (red), then co-cultured for 24 h. The arrows indicate that labeled mitochondria from hESC-MSCs appeared in HGFs. Scale bar, 100 μm (upper) and 25 μm (lower). (e) Representative scatter chart of Group control and Group Mito. The transfer subset (FITC+/ PE+) cells were detected by flow cytometry (n=3). (f) Relative mRNA expression of NLRP3, IL-6, IL-1β, and TNF-α. (g) The protein expression levels of NLRP3, GSDMD, Caspase-1, IL-1β, and β-actin were measured by western blot. Data are presented as means ± SEM. Unpaired two-tailed Student's t-test were conducted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Mito: cultured with mitochondria; IC: indirectly co-cultured; LPS: lipopolysaccharide; ATP: adenosine triphosphate; MFI: median fluorescence intensity; ROS: reactive oxygen species; NLRP3: NOD-like receptor family pyrin domain-containing 3; GSDMD: Gasdermin D; SEM: standard error of mean.

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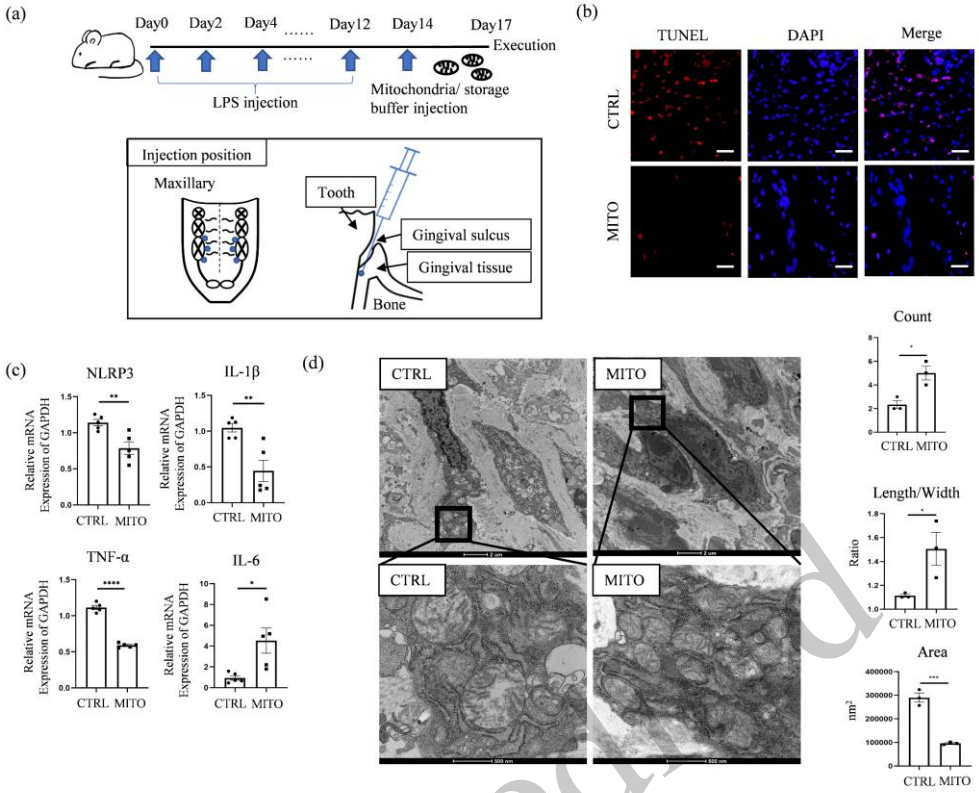

Figure 5 Mitochondria derived from hESC-MSCs enhanced the recovery of experimental periodontitis in mice. (a) Diagram of the animal experimentation process. The injection position was in the palatal gingival sulcus of the first maxillary molar, which was indicated by the blue note. (b) Gingival tissue sections stained with TUNEL and DAPI. Scale bar, 20 μm. (c) Relative mRNA expression of NLRP3, IL-1β, and TNF-α (n=5). (d) The substructure of mitochondria of gingival tissues observed by TEM. The mitochondria from Group CTRL show swelling, enlargement and loss of cristae. The mitochondria from Group MITO are smaller and have a larger aspect ratio. The double membrane structure and cristae are clear. Scale bar, 2 μm (upper) and 500 nm (lower). The mitochondrial count, length/width ratio, and area were calculated (n=3). Data were presented as means ± SEM. Unpaired two-tailed Student's t-tests were conducted. *p < 0.05, **p < 0.01, ***p < 0.001. DAPI: 4',6-diamidino-2-phenylindole; TUNEL: TdT-mediated dUTP nick end labeling; TEM: transmission electron microscopy; NLRP3: NOD-like receptor family pyrin domain-containing 3; SEM: standard error of mean.

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