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Phosphatase and tensin homologue determine inflammatory status by differentially regulating the expression of Akt1 and Akt2 in macrophage alternative polarization of periodontitis

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Abstract

Aim: Macrophages are closely involved in periodontitis. However, the molecular mechanism by which macrophages influence periodontitis is not well understood. We investigated the effects of phosphatase and tensin homologue (PTEN) on macrophage polarization, the underlying mechanism and the regulatory roles in periodontium regeneration.

Materials and Methods: PTEN expression in periodontitis macrophages was detected ex vivo. The effects of PTEN on macrophage polarization and the underlying mechanisms were investigated in vitro. We also analysed the ability of PTEN inhibitors to repair periodontitis in vivo in a ligature-induced mouse model of periodontitis.

Results: Macrophage PTEN expression in periodontitis patients was significantly higher than that of controls. PTEN inhibition in macrophages induced alternative macrophage polarization, whereas PTEN overexpression facilitated classical polarization. PTEN inhibition facilitated activation of Akt1 while inhibiting expression of Akt2. Furthermore, Akt2 overexpression could rescue the effects of PTEN inhibition on NF-κB. Treatment with a PTEN inhibitor significantly attenuated the local inflammatory status and prevented alveolar bone resorption in the mouse model.

Conclusions: Our findings suggest that PTEN inhibition could induce alternative macrophage polarization by differentially regulating Akt1 and Akt2. This also changed a pro-inflammatory microenvironment to an anti-inflammatory environment by subsequently regulating the expression of NF-κB, thereby attenuating inflammatory alveolar bone resorption induced by ligature.

KEYWORDS
Akt1, Akt2, macrophage polarization, periodontitis, PTEN

Clinical Relevance

Scientific rationale for study: Identifying an efficient strategy to reverse a pro-inflammatory microenvironment in periodontitis is important as this can relieve the severity and complications of periodontitis. Macrophage polarization may affect the inflammatory microenvironment in periodontal tissue. The effects of phosphatase and tensin homologue (PTEN) on macrophage polarization in periodontitis and the underlying molecular mechanisms remain elusive.

Principal findings: PTEN inhibition induced alternative macrophage polarization by activating Akt1 while inhibiting Akt2. The differential regulation of Akt1 and Akt2 further influenced

NF-κB and the inflammatory micro-environment of the periodontium. Applying a PTEN-targeted inhibitor promoted regeneration of periodontal defects.
Practical implications: We highlighted PTEN inhibition as a potential candidate for immunoregulation and regeneration of periodontal defects.

1 | INTRODUCTION

Periodontitis is an inflammatory disease that induces progressive tooth attachment loss and alveolar bone resorption and is closely related to systemic diseases (Chukkapalli et al., 2018; Renn et al., 2018). As the main component of innate immunity, macrophages have been proven to play non-negligible roles in the progression and recovery of periodontal inflammation (Yang et al., 2014). Macrophages can be polarized to the classical phenotype, in which state they play a pro-inflammatory role. Most studies have shown that classical macrophages, which highly express interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS), are predominant in periodontitis (Hussain et al., 2016). However, macrophages can also be polarized to an alternative phenotype. Some studies have indicated that markers of alternatively polarized macrophages, which exhibit anti-inflammatory effects and promote tissue regeneration, are also increased in periodontitis (Gheren et al., 2008; Navarrete et al., 2014). The ratio of macrophages with the classical phenotype with to those with the alternative phenotype has been proposed as a means to evaluate inflammatory status (Yang et al., 2018; Zhou et al., 2019). However, some studies have revealed that this ratio is significantly higher in periodontitis, while other studies have stated that the ratio in periodontitis is comparable to that in the healthy population (Garaicoa-Pazmino et al., 2019; Zhou et al., 2019). Our previous study found that the numbers of both classical and alternative phenotype macrophages were increased in periodontitis patients, although those with the classical phenotype were dominant over those with the alternative phenotype (X. Wu et al., 2020). This result was in line with most previous studies and supported the hypothesis that the induction of alternative macrophage polarization could locally modulate the microenvironment to an anti-inflammatory status and induce periodontal defect regeneration.

Phosphatase and tensin homologue (PTEN) is a critical regulator of tumorigenesis and bone remodelling. Recent studies have shown that PTEN expression is associated with periodontitis. PTEN inhibition promotes the proliferation and osteogenic differentiation of periodontal stem cells, thus maintaining homeostasis in the periodontium (Lv et al., 2020). Li et al. (2021) found that hsa_circ_0003948 regulates periodontitis via PTEN. Moreover, some researchers have proposed that pharmacological inhibition of PTEN could be applied to the treatment of tissue defects (Borges et al., 2020). In contrast, a previous study indicated that PTEN deficiency could promote inflammation and increase the number of osteoclasts, thus inducing alveolar bone resorption (C. Fu et al., 2019). Therefore, the relationship between PTEN and periodontitis remains controversial, and it is necessary to elucidate this relationship and its potential mechanism.

The effects of PTEN on macrophage polarization have also been investigated in recent years (Cheng et al., 2017). Accumulating evidence has indicated that PTEN can induce classical macrophage polarization via dephosphorylation of PIP3 to PIP2, which inhibits maintenance of homeostasis of the periodontal PI3K–AKT signalling pathway (Sahin et al., 2014). However, our previous study revealed that selective inhibition of Akt2 could induce alternative macrophage polarization (X. Wu et al., 2020). As previous studies have focused on the effects of PTEN on pan-Akt, our results raised a question about the relationship between PTEN and different Akt isoforms. We hypothesized that PTEN inhibition could activate Akt1 while inhibiting the activation of Akt2.

Accordingly, this study aimed to determine the influence of PTEN on macrophage polarization, its underlying mechanism and its effects on periodontitis, a common inflammatory disease. As previous studies have indicated that the NF-κB signalling pathway is closely related to the inflammatory environment of periodontitis (Golz et al., 2015), we investigated whether PTEN regulates NF-κB signalling via Akt2.

2 | MATERIALS AND METHODS

2.1 | Gingival sample collection

The study collected gingival tissues from patients seeking periodontal treatment at Peking University School and Hospital of Stomatology. The study was approved by the Biomedical Ethics Committee of Peking University and Hospital of Stomatology (PKUSSIRB-201951179). The inclusion and exclusion criteria are presented in Table 1. The criteria were formulated according to a previous report (Garaicoa-Pazmino et al., 2019). Gingival samples were fixed in 4% paraformaldehyde solution for 24 h, dehydrated and embedded in paraffin.

2.2 | Haematoxylin and eosin staining and immunological staining

The paraffin-embedded gingival specimens were cut into 5-μm sections for histological and immunostaining analyses. Haematoxylin and eosin (H&E) staining was performed according to the instructions of the H&E staining kit (G1120, Solarbio Science and Technology Pty, Ltd., Beijing, China). Immunohistochemistry and immunofluorescence staining were performed as described in our previous study (X. Wu et al., 2020). Antibodies against CD68 (Santa Cruz Biotechnology Inc., USA), CD68 (Affinity BioSciences, USA), PTEN (Affinity BioSciences), CD206 (Abcam, USA), CD86 (Affinity BioSciences), CD163 (Bioss Inc., USA), IL-1β (Servicebio, USA) and IL-10 (Santa Cruz Biotechnology

TABLE 1 Inclusion and exclusion criteria of gingival samples

Inclusion criteria		
Control	**Periodontitis**	**Exclusion criteria**
Probing depth <3 mm	Probing depth >6 mm	Cigarette smoking
Bleeding index ≤1 or BOP(−)	Bleeding index ≥2 or BOP(+)	Systematic diseases that might influence the periodontal condition;
No attachment loss	Attachment loss >3 mm	Anti-inflammatory medications intake during the past 3 months
No alveolar bone loss in radiographic examination	Obvious alveolar bone loss in radiographic examination	Periodontal treatment during the past 6 months
Teeth number ≥20	No limitation	Pregnant or breastfeeding

Abbreviation: BOP, bleeding on probing.

Inc.) were used for immunological staining. Images were acquired using a laser scanning confocal microscope (LSCM, Zeiss, Wetzlar, Germany).

2.3 | Cell culture and transfection

RAW 264.7 cells were cultured according to our previous study (X. Wu et al., 2020). Cells were treated with 1 μg/ml lipopolysaccharide (LPS; Sigma, St. Louis, MO, USA) for 12 h before being used in assays to induce classical macrophage classical polarization. To induce alternative macrophage polarization, cells were treated with 50 ng/ml IL-4 and 50 ng/ml IL-13 for 12 h.

Small interfering RNA (siRNA) targeting *Pten* or a plasmid carrying a *Pten* expression cassette, designed to knock down or overexpress *Pten*, respectively, were transfected into the cultured cells. Negative control siRNA (siNC) and empty vector were used as controls, respectively. The transfection experiments were processed according to the protocol recommended by the manufacturer of the transfection reagent (PT-114-15, jetPrime, Strasbourg, France).

2.4 | Flow cytometry

PTEN was knocked down or overexpressed in RAW 264.7 cells under different conditions, labelled by fluorescence-conjugated antibodies and then subjected to flow cytometry, according to the manufacturer's instructions and as previously published (X. Wu et al., 2020). FITC-F4/80 (Abcam), APC-CD86 (BioLegend, USA) and PE-CD206 (BioLegend) antibodies were used to characterize the classical and alternative macrophage polarization. APC-conjugated IgG2a (BioLegend), FITC-conjugated IgG2b (Abcam) and PE-conjugated IgG2a (BioLegend) were used as isotype controls to analyse macrophage polarization. Labelling was quantified using a BD FACS Aria III flow cytometer (Franklin Lakes, NJ, USA).

2.5 | Quantitative reverse-transcription polymerase chain reaction and Western blotting

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and western blotting were performed as described previously (X. Wu et al., 2018, 2020). The sequences of target-specific primers are listed in Appendix Table 1. For western blotting, antibodies included total P65 (t-P65), PTEN, Akt2 and GAPDH from Affinity BioScience (USA); phosphorylated-P65 (p-P65), phosphorylated-Akt2 and phosphorylated-pan-Aktfrom Cell Signalling Technologies (USA); and Akt1, p-Akt1 and Arginase-1 (Arg-1) from Santa Cruz.

2.6 | Mice ligature-induced periodontitis model

The animal experiments were performed by the Biomedical Ethics Committee of Peking University (No. LA2019092). Male C57BL/6 mice were randomly divided into the sham, periodontitis and periodontitis+SF1670 groups. After general anaesthesia, sterile silk ligatures were placed in the cervical region of the maxillary second molar. In the sham group, the ligatures were immediately removed. For the periodontitis and periodontitis+SF1670 groups, ligatures were kept at the site for a week. SF1670 was injected locally in the periodontal region of the periodontitis+SF1670 group, while physiological saline was injected in the sham and periodontitis groups. The mice were euthanized and the maxillae were removed and fixed in a 4% paraformaldehyde solution for 24 h. Fixed mouse maxillae were scanned using an animal micro-computed tomography (CT) scanner (Bruker, Belgium). This study conformed to the ARRIVE 2.0 (Animal Research: Reporting of In Vivo Experiments [ARRIVE]) guidelines.

2.7 | Statistical analysis

Numerical data are expressed as the mean ± standard deviation. Statistical differences were compared using one-way analysis of variance (ANOVA) and Tukey's test (SPSS 13.0, IBM SPSS Inc., Armonk, NY, USA). Differences were considered statistically significant at $p < .05$. The experiments were repeated at least three times.

3 | RESULTS

3.1 | Higher PTEN expression in the macrophage of periodontitis

Immunohistochemistry staining of CD68, CD86 and CD206 was performed to characterise the infiltration of total macrophage, classical

polarized macrophage and alternative polarized macrophage. Results showed that the counts of total macrophage, classical polarized macrophage and alternative polarized macrophage were higher in the periodontitis group than in the control group, while the augmentation of classical polarized macrophage was more prominent than alternative polarized macrophage (Appendix Figure 1). To investigate the expression profile of PTEN in the macrophages of periodontitis cases, gingivae from healthy individuals and periodontitis patients were collected, and H&E staining and immunofluorescent co-staining of PTEN and CD68 were performed. H&E staining revealed that the infiltration of inflammatory cells was more significant in the periodontitis group than in the control group (Figure 1a). Moreover, fluorescent co-staining revealed more macrophage infiltration and higher expression of PTEN in macrophages from periodontitis patients (Figure 1b). Statistical results indicated higher percentages of $PTEN^{+}CD68^{+}$ cells in $CD68^{+}$ cells of the periodontitis group than control, and a higher mean fluorescent density of PTEN in macrophage of the periodontitis group than control (Figure 1c). The co-localization of CD68 and PTEN indicated that aberrantly high expression of PTEN in macrophages might be involved in the regulation of the local inflammatory microenvironment of periodontitis, thus influencing the progression of periodontitis.

3.2 | PTEN interruption influences the phenotypes of macrophages both under pro-inflammatory and anti-inflammatory environments

PTEN was knocked down by siRNA or was overexpressed using plasmids carrying PTEN expression cassettes. Flow cytometry revealed that PTEN inhibition significantly increased the percentage of $F4/80^{+}CD206^{+}$ cells and decreased the percentage of $F4/80^{+}CD86^{+}$ cells under unstimulated conditions. Moreover, PTEN overexpression reversed these effects (Figure 2a). These results indicated that PTEN inhibition could induce alternative macrophage polarization while PTEN overexpression induced classical macrophage polarization. Thereafter, we investigated whether the effects of PTEN remained the same in anti-inflammatory (IL-4- and IL-13-stimulated) and pro-inflammatory (LPS-stimulated) environments.

Flow cytometry results revealed that, in a pro-inflammatory environment, PTEN inhibition significantly increased the percentage of $F4/80^{+}CD206^{+}$ cells and decreased the percentage of $F4/80^{+}CD86^{+}$ cells. However, PTEN overexpression adversely affected macrophage polarization (Figure 2b). Statistical analysis of flow cytometry results showed that, under both unstimulated and pro-inflammatory conditions, PTEN inhibition significantly reduced

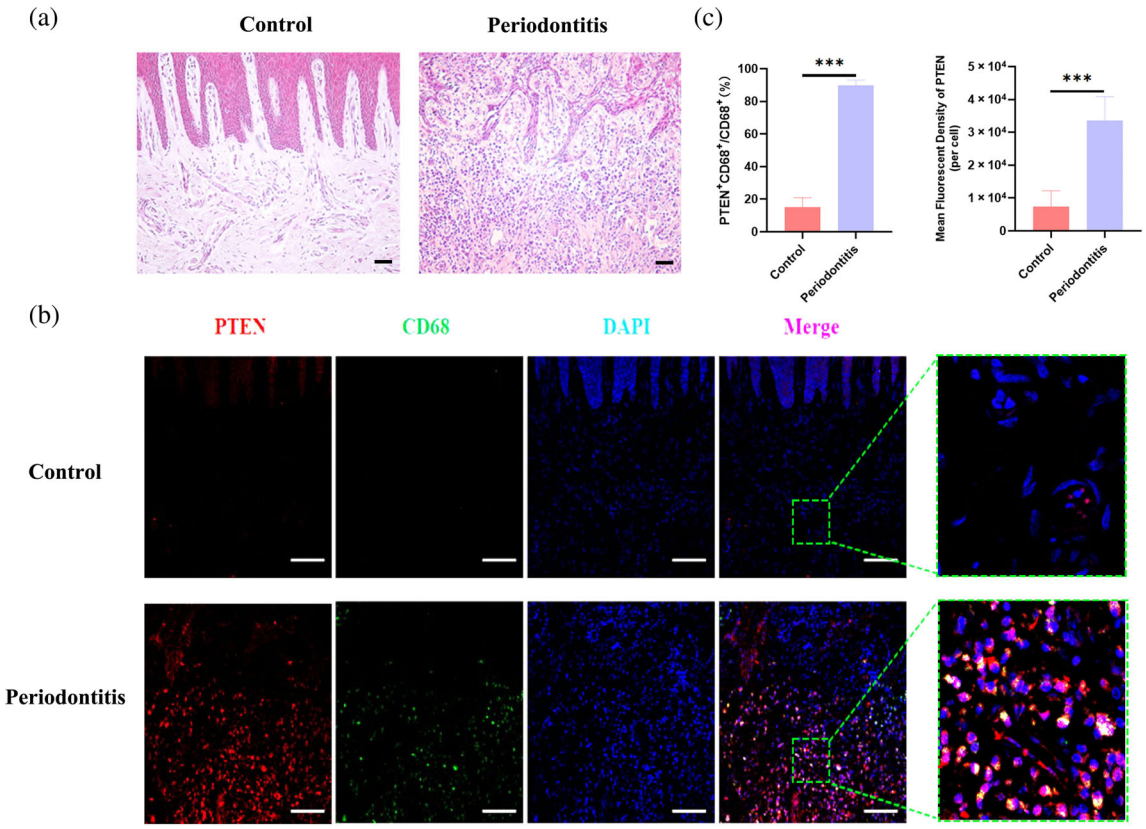

FIGURE 1 (a) Haematoxylin and eosin staining of the gingivae of periodontitis patients and healthy controls (scale bar = 100 μm). (b) Representative images showing colocalization of phosphatase and tensin homologue (PTEN) (red) and CD68 (green) in healthy gingivae and those with periodontitis. Scale bar = 100 μm. (c) Quantitative analysis of the percentages of PTEN positive cells in macrophage (left) and mean fluorescent density of PTEN in each macrophage (right) in periodontitis and control groups. $p < .05$ represents a significant difference, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

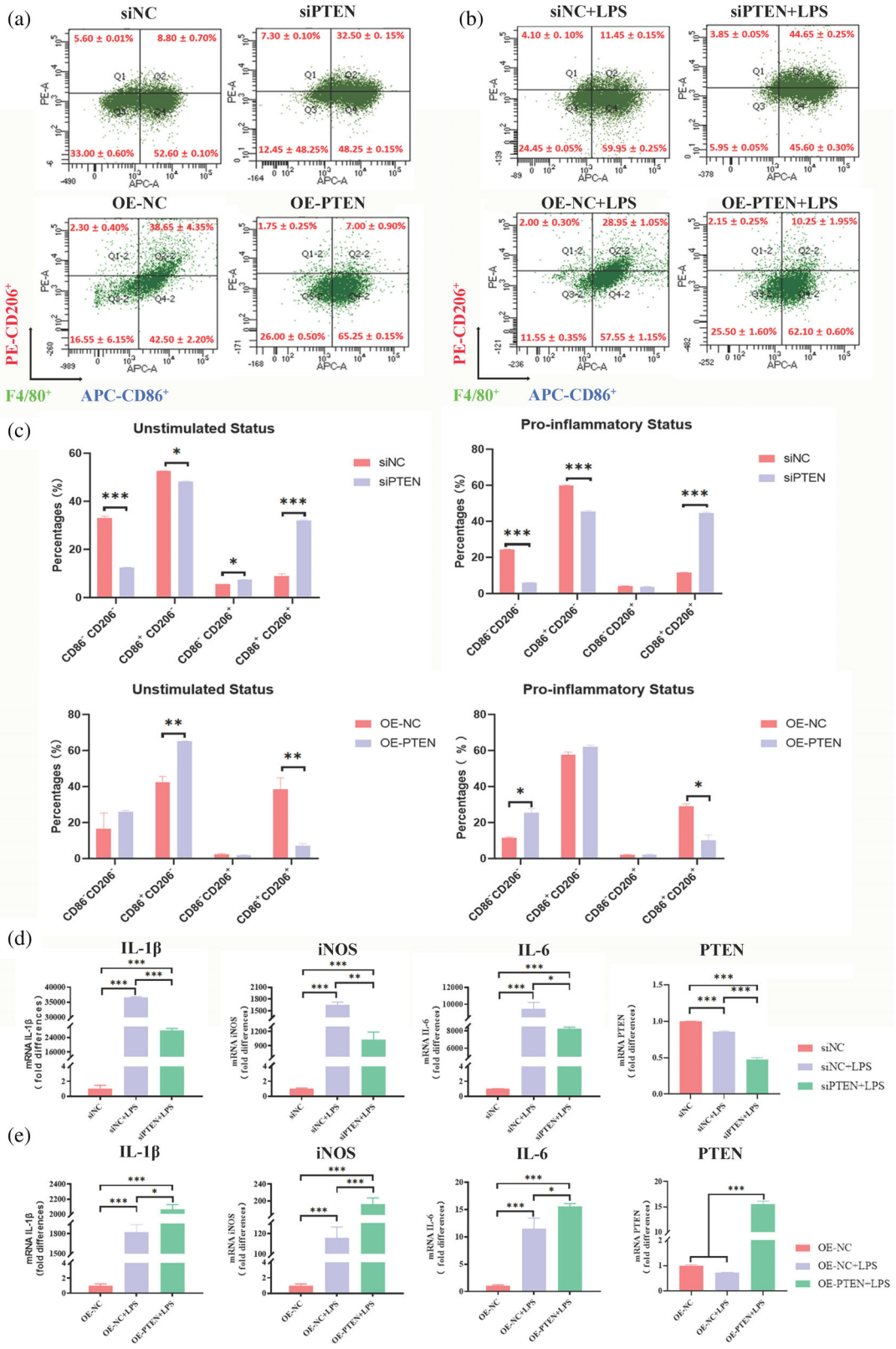

FIGURE 2 APC-CD86, PE-CD163 and FITC-F4/80 were stained for flow cytometry analysis without (a) and with lipopolysaccharide (LPS)-induced pro-inflammatory conditions (b). (c) Statistical results of flow cytometry. $p < .05$ represents a significant difference. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. phosphatase and tensin homologue was knocked down (d) or overexpressed (e) and quantitative reverse-transcription polymerase chain reaction was used to evaluate pro-inflammatory genes, including interleukin-1β, inducible nitric oxide synthase and interleukin-6. $p < .05$ represents a significant difference, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

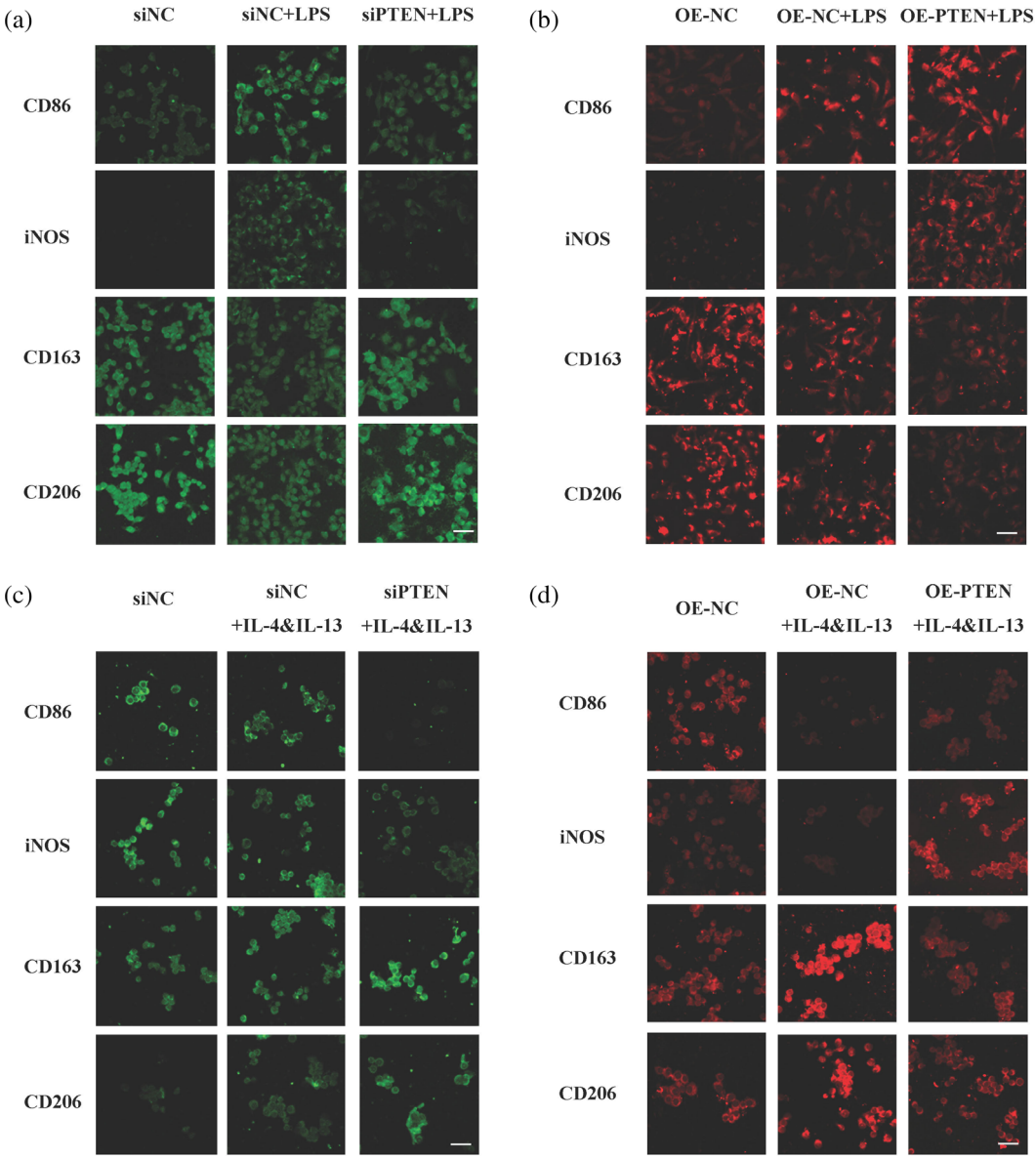

FIGURE 3 In a pro-inflammatory environment, with lipopolysaccharide stimulation, phosphatase and tensin homologue (PTEN) was knocked down (a) or overexpressed (b). Immunofluorescent staining of CD86, inducible nitric oxide synthase (iNOS), CD163 and CD206 was performed to evaluate the polarization of macrophages. Scale bar = 50 μm. In an anti-inflammatory environment, with interleukin (IL)-4 and IL-13 stimulation, PTEN was knocked down (c) or overexpressed (d). Immunofluorescent staining of CD86, iNOS, CD163 and CD206 was performed to evaluate the polarization of macrophages. Scale bar = 50 μm

the percentages of $F4/80^{+}CD86^{-}CD206^{-}$ and augmented the percentages of $F4/80^{+}CD86^{+}CD206^{+}$ macrophages, but not that of $F4/80^{+}CD86^{-}CD206^{+}$ macrophages. These results implied that PTEN inhibition might induce a change from classical macrophage polarization to alternative polarization directly or might induce indirect polarization of naïve macrophages to an alternative phenotype, by first transforming naïve macrophages to the classical phenotype and then further polarizing them to an alternative phenotype. PTEN overexpression further validated this hypothesis by revealing a marked decrease in $F4/80^{+}CD86^{+}CD206^{+}$ macrophages (Figure 2c).

Fluorescent staining was performed to detect CD86 and iNOS, which are associated with classical polarization, and CD163 and CD206, which are associated with alternative polarization. The results showed that PTEN inhibition markedly decreased the expression of CD86 and iNOS and increased the expression of CD163 and CD206 (Figure 3a). In contrast, PTEN overexpression exhibited the opposite effects (Figure 3b). qRT-PCR was performed to observe the expression of pro-inflammatory genes, including IL-1β, iNOS and IL-6. The results revealed that PTEN inhibition significantly decreased the expression of proinflammatory genes (Figure 2d). PTEN overexpression exhibited the opposite effects, which further promoted the expression of pro-inflammatory genes (Figure 2e). Therefore, even under pro-inflammatory conditions, PTEN inhibition can induce alternative polarization of macrophages and significantly decrease the expression of pro-inflammatory genes.

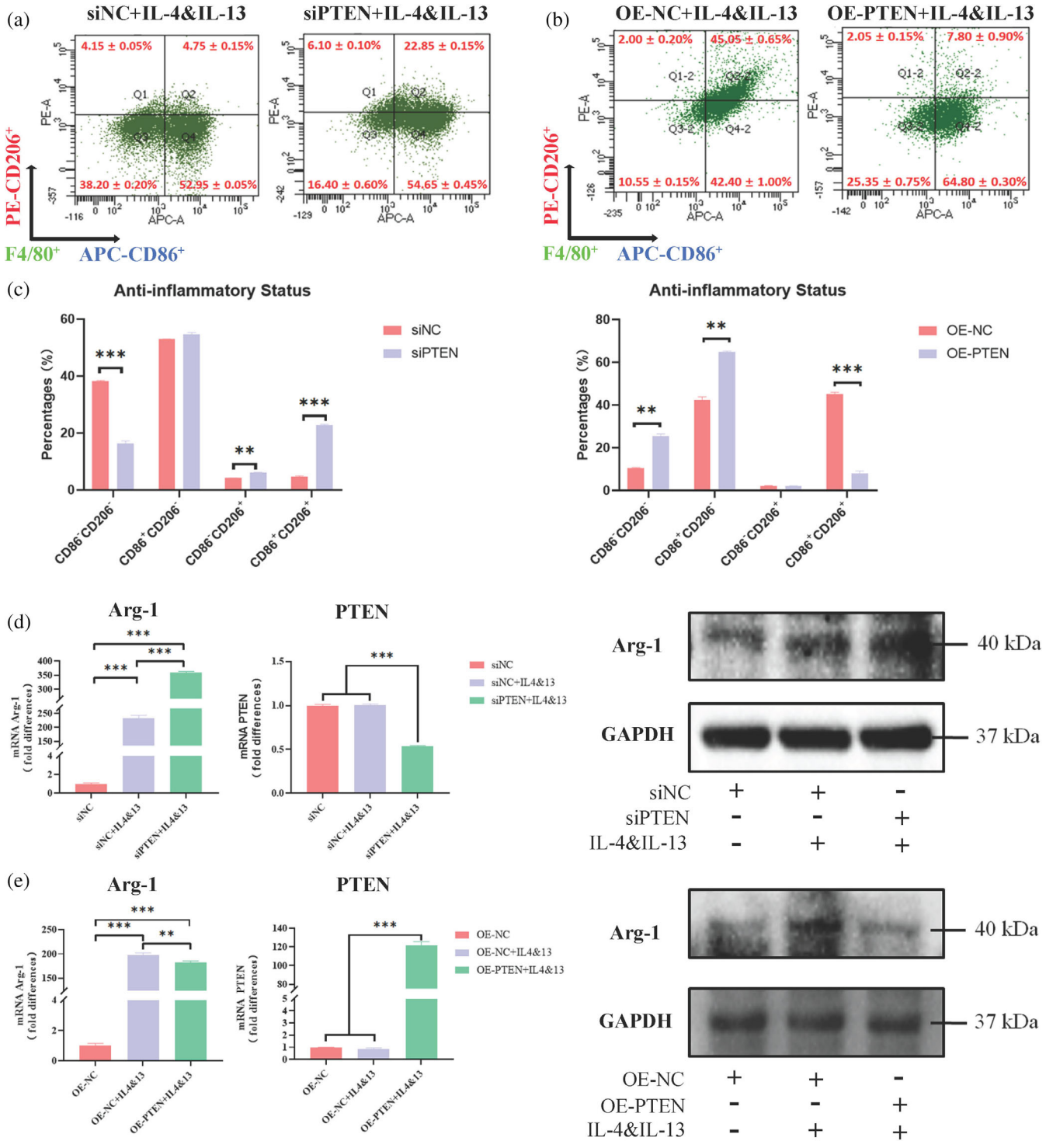

FIGURE 4 In an anti-inflammatory environment and with phosphatase and tensin homologue (PTEN) knocked down (a) or overexpressed (b), APC-CD86, PE-CD163 and FITC-F4/80 were stained for flow cytometry analysis. (c) Statistical results of flow cytometry are shown. $p < .05$ represents a significant difference. *$p < .05$; **$p < .01$; ***$p < .001$. PTEN was knocked down (d) or overexpressed (e) and quantitative reverse-transcription polymerase chain reaction and western blotting were processed to evaluate the expression of ARG1. $p < .05$ represents a significant difference, *$p < .05$; **$p < .01$; ***$p < .001$.

In an anti-inflammatory environment, flow cytometry revealed that PTEN inhibition significantly increased the percentage of $F4/80^{+}CD86^{+}$ $CD206^{+}$ cells and decreased the percentage of $F4/80^{+}CD86^{-}$ $CD206^{-}$ cells (Figure 4a), whereas PTEN overexpression exhibited adverse effects (Figure 4b). PTEN inhibition significantly increased the percentage of $F4/80^{+}CD86^{+}CD206^{+}$ macrophages. PTEN overexpression showed

adverse results that were in line with an unstimulated and pro-inflammatory status (Figure 4c). Fluorescent staining revealed that PTEN inhibition significantly increased CD163 and CD206 expression while decreasing CD86 and iNOS expression (Figure 3c). PTEN overexpression resulted in adverse effects (Figure 3d).

Expression of Arg-1, a key marker of alternative macrophage polarization, was examined using qRT-PCR and western blotting. The results indicated that IL-4 and IL-13 increased the expression of Arg-1, while PTEN inhibition further increased its expression (Figure 4d). In contrast, PTEN overexpression decreased the expression of Arg-1 (Figure 4e). Collectively, similar to the unstimulated and pro-inflammatory state, PTEN inhibition could promote alternative macrophage polarization in an anti-inflammatory environment.

Overall, PTEN inhibition induced alternative macrophage polarization, while PTEN overexpression promoted classical macrophage polarization. It is worth noting that our results supported our hypothesis that PTEN inhibition could induce alternative macrophage polarization directly by inducing a change in the phenotype of classical macrophages or indirectly by inducing naïve macrophages to take on an alternative phenotype via the classical phenotype.

3.3 | PTEN regulates NF-κB signalling pathway via differentially modulating Akt1 and Akt2

The NF-κB signalling pathway has been proven to be critical to the progression of periodontitis (Golz et al., 2015); thus, we investigated the influence of PTEN on the NF-κB signalling pathway. In a pro-inflammatory environment, PTEN inhibition significantly decreased p-P65 expression while overexpression of PTEN facilitated its expression (Figure 5a and Appendix Figure 2). The effects of PTEN on P65 were verified in an anti-inflammatory environment (Figure 5b and Appendix Figure 3). The above data clearly indicated that PTEN could influence the activation of NF-κB.

Previous studies have indicated that, similar to PTEN, Akt2 could regulate the activation of the NF-κB signalling pathway (Ghosh et al., 2017). Therefore, we hypothesized that PTEN regulates the NF-κB signalling pathway via Akt2. First, we explored the effects of PTEN on different Akt isoforms, mainly pan-Akt, Akt1 and Akt2. Our results revealed that, under both pro-inflammatory and anti-inflammatory conditions, PTEN inhibition facilitated the phosphorylation of pan-Akt and Akt1. In contrast, PTEN inhibition decreased total and phosphorylated

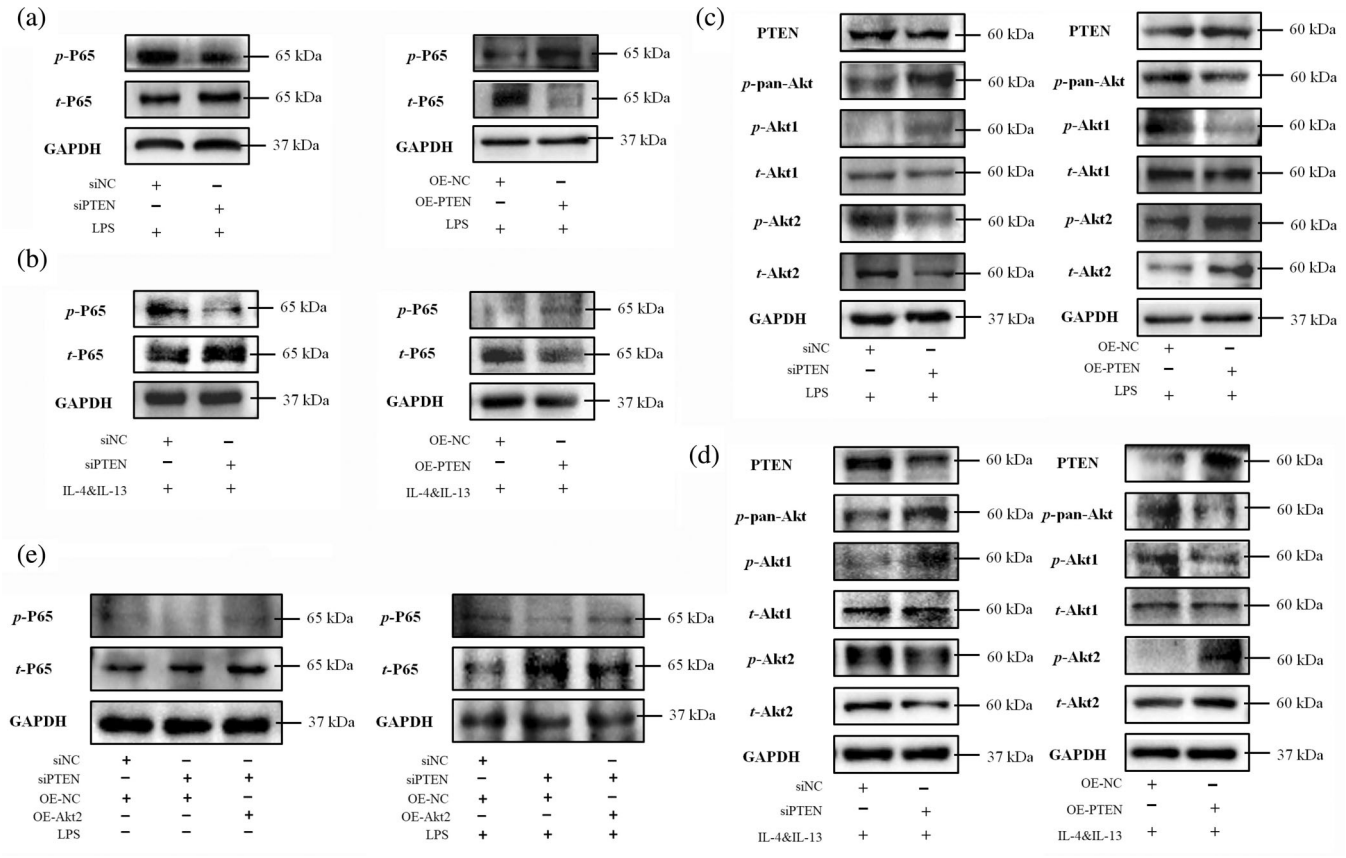

FIGURE 5 Phosphatase and tensin homologue (PTEN) inhibition could promote activation of Akt1 and inhibit the expression and activation of Akt2, thereby inhibiting activation of the NF-κB signalling pathway. (a) In a pro-inflammatory environment, PTEN knockdown could inhibit the activation of P65, while PTEN overexpression exhibited converse effects. (b) In an anti-inflammatory environment, PTEN interference exhibited similar effects on NF-κB signalling pathway as in the pro-inflammatory environment. Western blotting results indicated that PTEN could differentially regulate the expression of different isoforms of Akt, in both a pro-inflammatory (c) and an anti-inflammatory (d) environment. (e) Akt2 overexpression could rescue the inhibitory effects of PTEN inhibition on the P65–NF-κB signalling pathway.

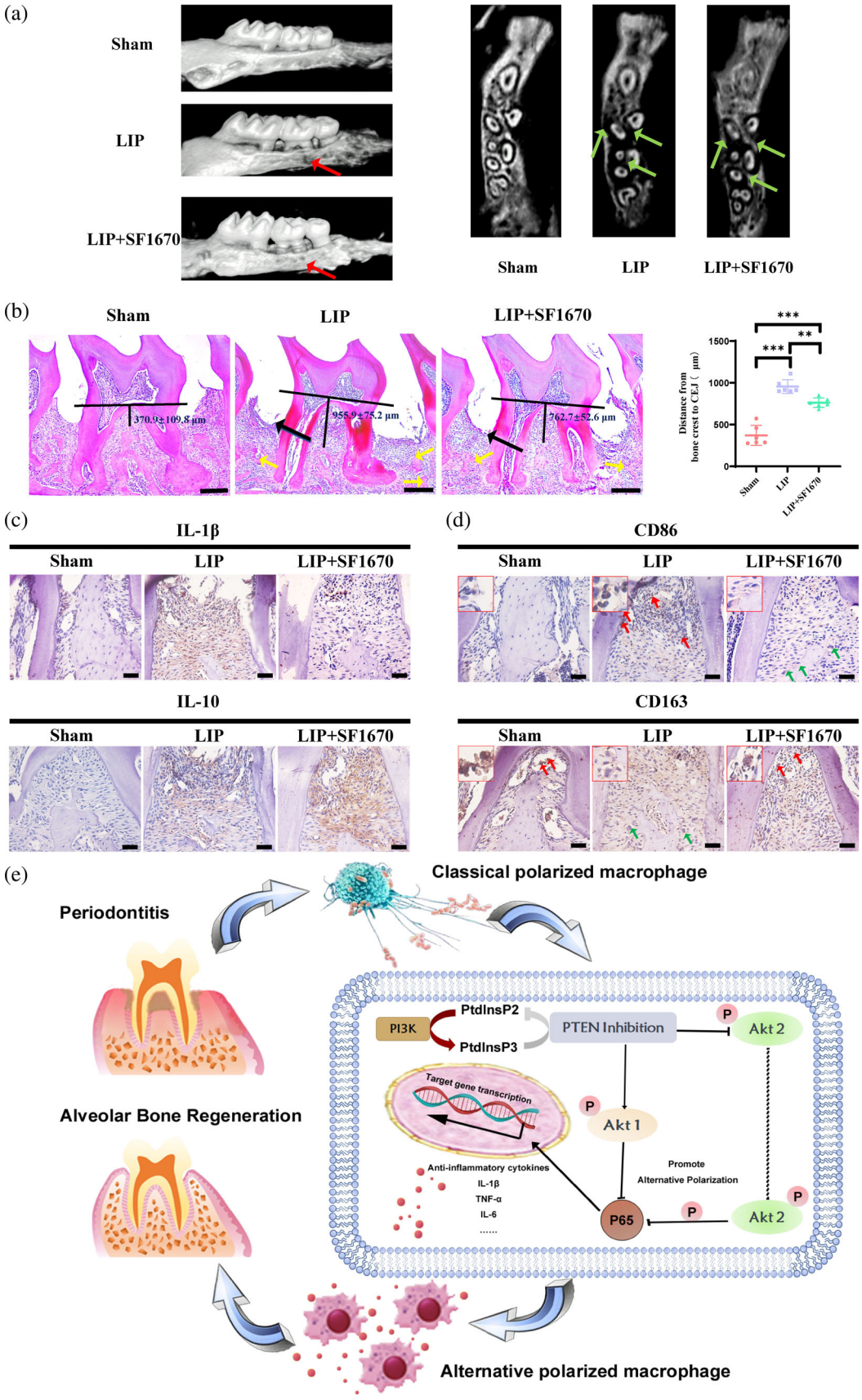

FIGURE 6 Legend on next page.

Akt2 levels. PTEN overexpression resulted in converse effects (Figure 5c,d and Appendix Figure 4). Thus, we showed differential regulatory effects of PTEN on different isoforms of Akt.

Next, we performed a rescue study, which indicated that, in both unstimulated and LPS-stimulated environments, Akt2 overexpression could rescue the effects of PTEN inhibition on the NF-κB signalling pathway (Figure 5e and Appendix Figure 5).

Collectively, PTEN inhibition facilitated activation of pan-Akt and Akt1, while decreasing the expression and activation of Akt2. Furthermore, PTEN regulated NF-κB by manipulating the expression of Akt2.

3.4 | PTEN inhibition facilitates alternative macrophage polarization and modifies the local inflammatory microenvironment of ligature-induced periodontitis

A model of ligature-induced periodontitis (LIP) was established, and SF1670, an inhibitor of PTEN, was applied to manipulate the inflammatory environment and prevent alveolar bone resorption. Micro-CT results showed that the silk ligature induced alveolar bone resorption at the mesial, distal and furcation regions of the maxillary second molar. The alveolar bone volume at the furcation and proximal regions was higher in the SF1670-treated group (Figure 6a). H&E staining showed obvious alveolar bone resorption in the LIP group. Empty lacunae were observed in the proximal regions of the LIP group, which were not obvious in the LIP+SF1670 group. The distance between the alveolar bone at the furcation region and the cementoenamel junction was measured. The distances in the Sham, LIP and LIP+SF1670 groups were 370 ± 109.8 μm, 955 ± 75.2 μm and 762.7 ± 62.6 μm, respectively. Moreover, the infiltration of inflammatory cells in the LIP group was obvious, whereas the infiltration was significantly decreased in the LIP+SF1670 group (Figure 6b). Immunohistochemical staining of IL-1β showed that LIP promoted the expression of the inflammatory cytokine IL-1β, while SF1670 attenuated its expression. Furthermore, levels of the anti-inflammatory cytokine IL-10 were significantly increased when SF1670 was applied (Figure 6c). The expression of $CD86^+$ macrophages was marked in the LIP group, whereas the expression of CD86 in the LIP+SF1670 group was not obvious. In contrast, $CD163^+$ macrophages were rarely found in the LIP group but increased significantly in the SF1670 treatment group (Figure 6d). The collected data indicated that PTEN inhibition could induce alternative macrophage polarization, change an adverse local pro-inflammatory microenvironment to an anti-inflammatory microenvironment and alleviate alveolar bone resorption in periodontitis.

4 | DISCUSSION

The current study found aberrantly high expression of PTEN in macrophages associated with periodontitis cases. Our results indicated that PTEN inhibition could directly induce alternative macrophage polarization from classical polarized macrophages, or indirectly by inducing naïve macrophages to polarize to the classical phenotype, and then further polarize to the alternative phenotype. The effects of PTEN inhibition not only alleviated the pro-inflammatory phenotype of macrophages under LPS stimulation but also reversed the pro-inflammatory phenotype to an anti-inflammatory phenotype. We found that PTEN inhibition facilitated activation of Akt1 while inhibiting the expression and activation of Akt2. To the best of our knowledge, no previous study has elucidated the differential regulatory effects of PTEN on Akt1 and Akt2. Moreover, we uncovered the regulatory roles of a novel PTEN/Akt2/NF-κB signalling pathway in macrophage polarization and in the regulation of the inflammatory environment. The PTEN inhibitor, SF1670, was applied for the treatment of periodontitis, which revealed that PTEN inhibition could alleviate the infiltration of inflammatory cells, regulate alternative macrophage polarization and attenuate alveolar bone resorption.

A recent study revealed the non-negligible effects of PTEN on macrophage polarization, with similar results to ours, that is, that PTEN inhibition induced alternative macrophage polarization. However, the underlying molecular mechanisms are not yet fully understood. The current study revealed a new PTEN/Akt2/NF-κB pathway in macrophage polarization. PTEN has long been recognized as an inhibitor of the Akt signalling pathway (Carnero et al., 2008), but previous studies have mainly focused on the activation of pan-Akt and Akt1. However, Akt has three isoforms, Akt1, Akt2 and Akt3 (Vergadi et al., 2017), thus it is essential to determine whether the effects of PTEN on different isoforms are similar. As Akt3 is mainly expressed in the brain, we only discuss the effects of PTEN on Akt1 and Akt2 in

FIGURE 6 A ligature-induced periodontitis model was constructed, and phosphatase and tensin homologue (PTEN) inhibition was applied as treatment for periodontitis. (a) Representative 3D reconstructive and horizontal section photos of mice in the Sham, ligature-induced periodontitis (LIP) and LIP+SF1670 group. Red arrows show the differences in the alveolar bone in 3D reconstructive photos, and green arrows show the differences in horizontal sections. (b) Haematoxylin and eosin staining showed that, compared with the LIP+SF1670 and Sham groups, more lacuna-containing bones (red arrows) and more inflammatory cell infiltration (black arrows) were found in the LIP group. The distance between the crest of the alveolar bone at the furcation and cemento-enamel junction was analysed. $p < .05$ represents a significant difference. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$. Scale bar = 500 μm (c) immunohistochemistry staining of interleukin (IL)-1β and IL-10 in the sham, LIP and LIP+SF1670 groups. Scale bar = 100 μm. (d) Immunohistochemistry of CD86 and CD163 in the sham, LIP and LIP+SF1670 groups. Red arrows indicated positively stained macrophages, and green arrows indicated negatively stained macrophages. Scale bar = 100 μm. (e) The diagram illustrates that PTEN inhibition might induce alternative macrophage polarization via the PTEN/AKT2/NF-κB signalling pathway, and reverse the locally pro-inflammatory microenvironment to an anti-inflammatory microenvironment, creating a suitable environment for the treatment of periodontitis.

this study. Our results implied that PTEN had an opposite regulatory role on Akt1 and Akt2. PTEN inhibition could facilitate activation of Akt1 and pan-Akt, which was in line with previous results that revealed that PTEN inhibited pan-Akt and Akt1, thus influencing activation of the NF-κB signalling pathway (T. Wu et al., 2015; Wan et al., 2021). In contrast, our study showed that PTEN inhibition could inhibit the expression of Akt2, thus influencing activation of the downstream NF-κB signalling pathway.

Although a series of studies indicated that PTEN inhibition could induce alternative macrophage polarization, there were still contradictions regarding the regulatory effects of PTEN on inflammation. Some researchers have suggested that PTEN upregulation inhibits inflammation and osteoclastic effects (Wang et al., 2020). Other researchers believed that PTEN inhibition ameliorated inflammation and promoted tissue regeneration (Zhang et al., 2019). The accumulated studies have spurred research into the regenerative effects of PTEN inhibitors on defects. H. Y. Fu et al. (2020) demonstrated that the PTEN inhibitor SF1670 could inhibit apoptosis and inflammation and could protect patients from intervertebral disc degeneration. Unfortunately, limited research has been conducted on periodontitis. A study on periodontitis indicated that PTEN overexpression could decrease the secretion of pro-inflammatory factors (C. Fu et al., 2019). However, Lv et al. (2020) demonstrated that PTEN inhibition could activate the Akt signalling pathway and promote periodontal stem cell proliferation and osteogenic differentiation. Our results were consistent with a later opinion, which indicated that PTEN inhibition could decrease the infiltration of inflammatory cells in periodontitis. Moreover, the secretion of pro-inflammatory IL-1β decreased while the secretion of anti-inflammatory IL-10 increased in the LIP+SF1670 group. In addition, the application of PTEN inhibitor also attenuated the tissue defects induced by silk ligature. The differences between studies might be induced by different kinds of macrophages, passages, or culturing environments.

Collectively, knockdown of PTEN in macrophages can induce alternative macrophage polarization and change an adverse pro-inflammatory state to an anti-inflammatory state. PTEN regulates macrophage polarization via the Akt2/NF-κB axis. However, contradictory results from different studies imply that the effects might be sensitive and specific to different cells or stimuli. Moreover, the current study is processed in RAW 264.7 cell lineage, which should be further validated in primary macrophage in the further to estimate the universality of results. This study focused on the effects of PTEN on macrophage, while its influence on periodontal ligament cells, bone marrow-derived stem cells and gingival fibroblast cells should also be investigated to illuminate the roles of PTEN in tissue repair and regeneration.

5 | CONCLUSION

In this study, we deduced the differential regulatory effects of PTEN on Akt1 and Akt2 expression. We revealed that PTEN knockdown inhibited the expression of Akt2 and subsequently influenced the activation of NF-κB, thus regulating alternative macrophage polarization and reversing the local pro-inflammatory microenvironment to an anti-inflammatory environment. The treatment effects of the PTEN inhibitor SF1670 on periodontitis further validated our findings that PTEN has regulatory effects on inflammation and macrophage polarization. These results highlight PTEN as a target for manipulating the inflammatory environment for the treatment of periodontitis.

AUTHOR CONTRIBUTIONS

Xiaowei Wu contributed to the conception and design, data acquisition, analysis and interpretation, drafted and critically revised the manuscript; Yidi Wang and Haotian Chen contributed to data acquisition and analysis, and critically revised the manuscript; Yixiang Wang and Yan Gu contributed to the conception and design, data analysis, drafted and critically revised the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to the authorship and/or publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The experimental protocol was approved by the Biomedical Ethics Committee of Peking University and Hospital of Stomatology (PKUSSIRB-201951179). Animal experiments were conducted by the Biomedical Ethics Committee of Peking University (No. LA2019092), as per the ARRIVE 2.0 (Animal Research: Reporting of In Vivo Experiments) guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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