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Roles of extracellular adenosine triphosphate on the functions of periodontal ligament cells

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OBJECTIVE: Adenosine triphosphate (ATP) is an essential nucleotide that is normally present in both intracellular and extracellular compartments. Extracellular ATP (eATP) has a pivotal role in both physiological and pathological processes of periodontal ligament tissues. Here, this review aimed to explore the various functions of eATP that are involved in the control of behaviours and functions of periodontal ligament cells.

METHODS: To identify the included publications for review, the articles were searched in PubMed (MEDLINE) and SCOPUS with the keywords of adenosine triphosphate and periodontal ligament cells. Thirteen publications were used as the main publications for discussion in the present review.

RESULTS: eATP has been implicated as a potent stimulator for inflammation initiation in periodontal tissues. It also plays a role in proliferation, differentiation, remodelling, and immunosuppressive functions of periodontal ligament cells. Yet, eATP has diverse functions in regulating periodontal tissue homeostasis and regeneration.

CONCLUSION: eATP may provide a new prospect for periodontal tissue healing as well as treatment of periodontal disease especially periodontitis. It may be utilized as a useful therapeutic tool for future periodontal regeneration therapy.

INTRODUCTION

Periodontal ligament cells (PDLCs) possess stem cells that have similar mesenchymal stem cell characteristic features. PDLCs can be differentiated into different cell types like cementoblasts, fibroblasts, and osteoblasts [1, 2]. As PDLCs have the ability to balance between new cell formation by proliferation and cell death, PDLCs have a cell renewal capacity [3]. Therefore, PDLCs may be the main cell source and a promising target approach for periodontal regeneration therapy. But its utilisation alone has some limitations; some conditions, like inflammatory environments, change the characteristic features of resident periodontal ligament cells [2, 4, 5]. Growth factors and molecular activities are required to stimulate resident PDLCs for effective periodontal regeneration. Many factors like VEGF, FGF2, IL1β, and IL12 participate in different periodontal regeneration stages to synergise periodontal regeneration and the regenerative ability [6–8]. Despite many factors and molecules involved in the periodontal regeneration process, we targeted adenosine triphosphate and explored its effects on PDLCs functions for this review.

Adenosine triphosphate (ATP) is an essential nucleotide and is normally found intracellularly and extracellularly. Both forms of ATP are involved in the physiological as well as pathological processes of various cell types. The amount of ATP in the extracellular environment during physiological conditions is relatively low [9]. Some conditions like mechanical stress induced the release of ATP into the extracellular environment by PDLCs [10–12]. The released ATP has different functions, such as proliferation, differentiation, and inflammatory response on different cell types. eATP induces proliferation through PKC, PI₃K/Akt, and MAPK signalling pathways in mouse embryonic stem cells [13]. It also acts as a danger signal by inducing the release of pro-inflammatory cytokines like IL1β in MG-5 microglial cells and IL6 in human thyrocytes [14, 15]. It has immunosuppressive action by stimulating IDO expression in the bone marrow mesenchymal stem cells (BMSCs) [16]. Different functions of PDLCs have been implicated in the periodontal regeneration process. However, the effects of eATP on the functions as well as behavior of PDLCs have not been extensively reviewed. This review aims to evaluate the various impacts of eATP on the functions and properties of PDLCs.

METHODS

The articles were searched in PubMed (MEDLINE) and SCOPUS databases using keywords without published period limitation for this review. The keywords used for the search are [adenosine triphosphate AND periodontal ligament cells]. The authors examined and evaluated the title and abstracts of the articles for inclusion and exclusion criteria. The inclusion criteria were as follows; (1) full-text articles published in English or other language articles with available English abstracts, (2) articles demonstrating

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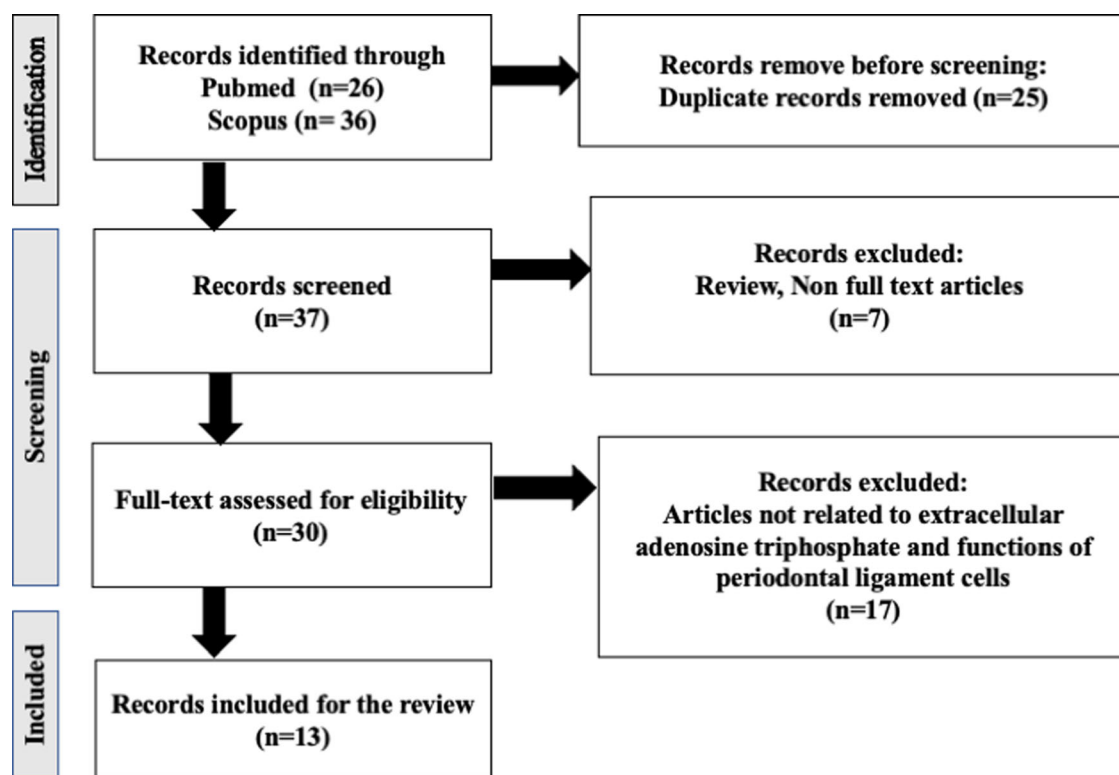
Fig. 1 Flow chart illustrating the article selection. Articles are selected for this review by using keywords; adenosine triphosphate (ATP) and periodontal ligament cells (PDLCs).

the effect of extracellular adenosine triphosphate related to functions of PDLCs, including inflammation, differentiation, immunomodulatory functions, and other functions of PDLCs. The exclusion criteria were (1) any study published in other languages, (2) articles related to the effect of adenosine triphosphate on other cell types rather than PDLCs (3) the study evaluating the effect of intracellular adenosine triphosphate.

RESULTS

37 original articles were found in search of the databases by using the described search procedure. According to inclusion and exclusion criteria, 7 non-English studies were removed, and we removed another 17 studies that are not related to extracellular adenosine triphosphate and functions of PDLCs. Final 13 studies were used for this review. We used Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) for the selection of literature for this review. The flow of information through the different steps involved in the selection of studies for this review (PRISMA) is shown in Fig.1.

Periodontal ligament cells (PDLCs)

The periodontal ligament is one of the tooth's supporting tissues and is involved in the periodontium together with the cementum and alveolar bone. It is a fibrous tissue rich in vascular supply and connects the tooth cementum on one side and the alveolar bone on either side. As it is a tooth-supporting tissue, it plays a crucial role in maintaining tooth stability and tuning biological functions. One of the periodontal ligament's major functions is maintaining periodontium homeostasis; for instance, it controls physiologic mechanical force during the masticatory function by transferring force to other supporting tissues of the tooth. The periodontal ligament can also play a key role in periodontal regeneration because it constitutes multiple cell types, such as cementum-forming cells (cementoblasts), bone-forming cells (osteoblasts),

nerve cells, fibroblasts, and vascular endothelial cells. Thereby, the periodontal ligament becomes a major cell source for maintaining periodontal tissue homeostasis and regeneration [1, 17]. Periodontal ligament cells (PDLCs) are present in periodontal ligament tissues, which are in periodontal ligament space. PDLCs own stem cells (progenitor cells) and have similar mesenchymal stem cell characteristics and features. They have multiple lineage differentiation abilities that can be transformed into cementoblasts, fibroblasts, osteoblasts, and adipose cells [1, 2, 18, 19]. Beside mesodermal lineages, stem cells isolated from periodontal ligaments can also differentiate into ectodermal and endodermal lineages [20, 21]. PDL fibroblasts are capable of renewal of cells that can be adjusted between new cell formation by proliferation and number of cell loss through cell death and cell migration [3].

Periodontal regeneration

The periodontal tissue regeneration process includes three consecutive phases: inflammation, proliferation, and remodelling. It is similar to other tissue healing processes [22]. However, some pathological and immunocompromised conditions like periodontitis imbalance the normal regeneration process that causes excessive and prolonged inflammatory phase, lack or delayed cell proliferation, and repairing phase leading to periodontal tissue destruction [23]. The final goal of periodontal regeneration is to form new functional periodontal tissue in place of the damaged periodontal tissue [24]. PDLCs may be the main cell source for periodontal regeneration by reason of their similar mesenchymal stem cell features like multilineage differentiation, proliferation, and self-renewal ability. In particular, PDLCs are easily accessible and expanded ex vivo [25]. Nevertheless, PDLCs could be used as a prioritized approach for regenerative treatment in various periodontal diseases. The success of regenerative treatment using PDLCs alone is very restricted because of some restrained conditions such as inflammatory conditions of host PDLCs

Table 1. Factors participate in the regulation of periodontal regeneration

No:	Factors	Functions	References
1.	VEGF	Enhances osteogenic differentiation by promoting alkaline phosphatase activity, mineralized structure formation and Runx2 expression in PDLSCs	[6]
2.	FGF2	Promotes progenitor cells proliferation	[6]
3.	rhBMP2	Regeneration of a mineralized layer mimicking dental cementum	[68]
4.	PDGF and TGFβ1	Enhances Periodontal healing by inducing PDLCs proliferation	[69]
5.	IL1β	Change LPS responsiveness PDLCs phenotype from osteoblastic characteristic phenotype	[7]
6.	IL6	Osteolytic factors and induces osteogenic differentiation	[70]
7.	IL12	Immunomodulatory function of PDLCs by inducing IFNγ, IDO, and HLA-G expression	[8]
8.	Poly I:C (TLR3 agonist)	Enhance the immunomodulatory properties of PDLCs by enhancing immunomodulatory molecules IFNγ, IDO	[71, 72]

[2, 4, 5]. Therefore, periodontal therapy needs other factors besides PDLC therapy for effective periodontal regeneration. Additional molecular activities are needed for the periodontal regeneration process by regulating in stages of the regeneration process; some molecules participate in cell proliferation, some in differentiation, some in control of the immune response, and some regulate the release of inflammatory mediators by PDLCs.

As a result of previous studies (as shown in Table 1), many factors participated in the stages of regeneration, in particular inflammation, immunoregulation, proliferation, differentiation, and maturation. Consequently, PDLCs possess stemness, proliferative, immunomodulatory, and differentiation properties; other factors boost the regenerative ability of PDLCs. Among many factors involved in the periodontal regeneration process, we emphasized the role of adenosine triphosphate in the functions of PDLCs in this review.

Adenosine triphosphate (ATP)

ATP is an essential nucleotide that is built up of a purine base (adenine), pentose sugar (ribose) and 3 phosphate groups. Adenine is attached to the carbon atom at 1'place, and 3 phosphate groups are attached at the 5' place of ribose. The 3 phosphate groups which are attached to the carbon atom at 1' place, are linked to ATP by high energy bonds. ATP is normally found in both intracellular and extracellular compartments. Intracellular ATP (iATP) functions as the intracellular energy source of the cells. eATP acts as an essential extracellular messenger [9]. Both iATP and eATP take part in physiologic and pathological conditions, involving different functions like inflammatory process, healing process, and immune responses of various cell types.

Functions of intracellular ATP (iATP)

iATP usage promotes skin wound healing resulting in granulation tissue formation, re-epithelialization, and increased VEGF release [26]. iATP promotes wound healing of rabbits' skin. It attracts the macrophage and inflammatory cells to the wound site and actuates the release of inflammatory cytokines: IL1β and TNFα, along with increases in VEGF expression, without the formation of hypertrophic scar in skin wounds of rabbits. Still, this study has limitations, and the detailed mechanism of the healing process may be specific to the species [27]. Mg-ATP encapsulated lipid vesicles treated at to wound site cause rapid granulation tissue regeneration, and new growth starts in less than 1 day [28]. These previous studies indicate the role of iATP in the wound healing process.

Functions of extracellular ATP (eATP) and its receptors

The amount of eATP is relatively low (400–100 nM) under physiologic conditions [9]. Many situations like mechanical stress, hypoxia, and inflammation cause the release of ATP into the extracellular compartment by various cell types such as PDLCs, cardiomyocytes, and endothelial cells [11, 12, 29, 30]. eATP has different functions, but it depends on various factors like cell types and types of activated receptors [31]. It acts as a danger signal called Danger-Associated-Molecular-Pattern Molecule (DAMP). It binds and activates the purinergic receptors on the cell surfaces, then initiates the inflammatory signal cascade and regulates the immune response [32, 33]. As the released ATP cannot be entered into the cell membrane easily to precede intracellular signalling events, it would rather interact with purinergic P_2 receptors on the cell surface.

Purinergic P_2 receptors are usually related to ATP. It has 2 subtypes according to signaling properties: metabotropic P_2Y receptors (P_2YRs) and inotropic P_2X receptors (P_2XRs). P_2Y receptors (P_2YRs) are classical G-protein-coupled receptors expressed in mammalian cells are eight subtypes (P_2Y_1, P_2Y_2, P_2Y_4, P_2Y_6, P_2Y_{11}, P_2Y_{12}, P_2Y_{13}, P_2Y_{14}). P_2Y receptors are activated by ATP, ADP, UTP, UDP, UDP glucose, and NAD (nicotinamide adenine dinucleotide). The intracellular signaling event of P_2Y_1, P_2Y_2, P_2Y_4, P_2Y_6, and P_2Y_{11} is related to PLC-IP$_3$R signaling pathway resulting in increased intracellular Ca^{2+} level. P_2Y_{12}, P_2Y_{13}, P_2Y_{14} receptors are mediated by AC-cAMP signaling pathway; ATP binds to these receptors leading to the inhibition of adenylyl cyclase (AC) and decreased intracellular cAMP levels [34, 35]. P_2X receptors (P_2XRs) are nucleotide-gated ion channel receptors with seven subtypes (P_2X_1, P_2X_2, P_2X_3, P_2X_4, P_2X_5, P_2X_6, P_2X_7) when ligand (nucleotide) gated ion channels P_2X receptors are activated by ATP. These are homo/hetero-trimers, eATP binds to receptors, ion channels opened, K^+ efflux and an influx of Ca^{2+} and Na^+ occur resulting in increased intracellular calcium and membrane depolarization [36, 37].

eATP gives different functions to different cell types; one of the dependent factors is the types of purinergic receptors. Different receptor activation by ATP influences many cell functions including proliferation, inflammation, immune response, and others. eATP is involved in the suppression of endometrial stem cell proliferation and migration [38]. eATP-P_2Y_1 receptor activation reduces the proliferation of BMSCs [39]. On the other hand, eATP could induce mouse embryonic stem cell proliferation through PKC, PI$_3$K/Akt, and MAPK signaling pathways. Therefore, eATP has different effects on cell proliferation in different environments through various signaling [13].

eATP plays a significant role in the control of inflammation of different cell types through diverse P_2 receptor interactions. In MG-5 microglial cell lines, eATP induces the maturation and release of IL1β by enhancing IL1β converting enzyme/ caspase [14]. eATP also stimulates other pro-inflammatory cytokine IL6 release in human thyrocytes that plays role in the control of thyroid function. eATP dose-dependently induced IL6 release through P_2Y receptor in human thyrocytes [15]. ATP can also induce anti-inflammatory cytokine IL10 expression and this

Table 2. Different functions of ATP according to types of cells and receptor activation

No:	Receptors	Mechanism	Functions of ATP	Cell types	References
1.	P_2	Immune response	Full activation of T reg cells	Naïve T reg cells	[51]
2.	P_2Y	Inflammation	Induces IL6 release	human thyrocytes	[15]
3.	P_2Y_1	Proliferation	Decreases proliferation in early passage of culture (P0-P5)	BMSCs	[39]
4.	P_2Y_1 P_2Y_{11}	Inflammation	Elevation of IL10	Macroglia cells	[40]
5.	P_2Y_{11}	Inflammation	decrease TNFα release and upregulate IL10 production	Human blood cells	[41]
6.	P_2Y_{11}	Immune response	Promotes T cells immunosuppression by upregulating IDO expression	IFNγ primed monocyte derived dendritic cells	[50]
7.	P_2Y_2 P_2Y_4	Tumor growth and progression	Induces ERK1/2, p38 and JNK1 phosphorylation	MCF-7 breast cancer cells	[46]
8.	P_2Y_2	Immune response	Enhances proliferation of T reg cells	T cells	[49]
9.	P_2X_3	Pain	Induces neurogenic inflammation	Human endometriotic cells	[48]
10.	P_2X_5	Inflammation	activates inflammasomes and IL1β secretion	Murine osteoclasts	[45]
11.	P_2X_7	Inflammation	Promotes the production of TNFα	LPS induced rat macroglia cells	[42]
12.	P_2X_7	Inflammation	Increases IL1β expression	*P.gingivalis* induced gingival epithelial cells	[43]
13.	P_2X_7	Inflammation	Upregulates IL6	LPS pretreated primary human skin fibroblasts	[44]
14.	P_2X_7	Immune response	1 mM ATP induces apoptosis and inhibits activated CD^{4+} T cell function	T cells	[49]
15.	P_2X_7	Immune response	Induces the release of IFNγ	Japanese flounder head kidney cells	[52]
16.	P_2X_7	Immune response	induces immunosuppression by increasing IDO expression	BMSCs	[16]
17.	Not specified	Proliferation and migration	Suppress proliferation and migration	Endometrial stem cells	[38]
18.	Not specified	Proliferation	Induces proliferation	Mouse embryonic stem cell	[13]
19.	Not specified	Inflammation	Induces the maturation and release of IL1β	MG-5 microglial cell lines	[14]
20.	Not specified	Immune response	250 nM ATP supports survival and proliferation of T lymphocytes	T cells	[49]

induction is related to the crosstalk between P_2Y_1 and P_2Y_{11} receptor activation in microglial cells. However, this induction effect is also dependent on intracellular Ca^{2+} release or the cAMP-activated PKA pathway [40]. In human blood cells, eATP-P_2Y_{11} interaction maintains the balance of inflammatory mechanism by increasing IL10 and decreasing TNFα [41]. P_2X_7 receptor activation induces TNFα release by LPS-induced microglial cells of rat [42]. This receptor activation also induces inflammation by different mechanisms in different cell types; upregulates IL1β expression by stimulating NALP3 inflammasome and caspase 1 in *P.gingivalis* induced gingival epithelial cells [43], promotes IL6 expression by LPS pretreated primary human skin fibroblasts [44]. Another P_2X receptor (P_2X_5) activation induces inflammasomes and IL1β secretion in murine osteoclasts [45].

Activation of P_2Y_2 and P_2Y_4 receptors by ATP regulates tumor growth and progression by inducing transcription factors, ERK1/2, p38 and JNK1 phosphorylation in MCF-7 cells [46]. eATP is involved in the regulation of human gingival tissue destruction by inhibiting IL1-induced matrix metalloproteinases (MMPs) expression via CD39 expression [47]. ATP-dependent P_2X_3 receptor activation increases endometrial pain by inducing neurogenic inflammation [48].

Various P_2 receptors activation by ATP imparts in an immune response. eATP plays a regulatory role in the immune response by adjusting specific CD^{4+} T cells response by activation of different P_2 receptors. 250 nM ATP upregulates the survival and proliferation of T lymphocytes by increasing the secretion of IL2. A higher dose 1 mM of eATP enhances apoptosis and inhibits activated CD^{4+} T cells functions through P_2X_7 receptor activation and enhances the proliferation of regulatory T cells by activation of P_2Y_2 receptors. Hence, the effect of eATP on specific CD^{4+} T cells response depends on the concentration of nucleotide [49]. eATP supports the immunoregulatory mechanism of dendritic cells. It inhibits Th_1 cytokine IL12 and stimulates Th_2 cytokines IL10. It also provides immunosuppressive action by inducing IDO expression through P_2Y_{11} receptor in monocyte-derived dendritic cells primed with IFNγ [50]. ATP-P_2 receptor activation has the full ability of immunosuppression by activating naïve T reg cells [51]. P_2X_7 receptor activation by 1000 µM eATP induces immunomodulatory cytokine IFNγ release in Japanese flounder head kidney cells [52]. eATP is involved in the immunosuppressive function of BMSCs primed with IFNγ by downregulating IDO expression via P_2X_7 receptor activation [16]. In conclusion, eATP has diverse functions of different cell subsets through various P_2 receptors. Different functions of ATP that depend on various cell types and different receptor activation are described in Table 2.

As stated in Table 2, eATP has diverse effects on various functions according to different receptor activation and cell types. PDLCs have different types of purinergic receptors on the cell surface. Many previous studies proved that eATP and P_2 receptor signalling had different functions on PDLCs.

Table 3. Roles of eATP in various functions of PDLCs.

No	Receptors	Mechanism	Functions of ATP	Conditions	References
1.		Proliferative action	Suppress PDLCs proliferation	PDLCs	[54]
2.	P_2X_7	Inflammatory chemokines	Induces IL8 and CCL 20 release	PDLCs	[55]
3.	P_2X_7	Pro-inflammatory cytokines	Induce release of IL1β	Mechanical stress induced PDLCs	[12, 56]
4.	P_2X_7	Immunosuppressive cytokines	Induces IDO and IFN γ expression	PDLCs	[57]
5.	P_2X_7	Osteogenic markers	Reduces osteogenic differentiation	Inflammatory mediated PDLCs	[58]
6.	P_2X_7	Osteogenic markers	Enhances Runx2 and OCN expression	PDLCs	[59]
7.	P_2Y_1	Osteogenic factor	Stimulates BMP-9 synthesis	Cyclic tensile stress induced PDLCs	[60]
8.	P_2Y_1	Osteoclast differentiation marker	Promotes RANKL expression through the P_2Y_1 cyclooxygenase pathway	Mechanical stress induced PDLCs	[61]
9.	P_2Y_1	Bone resorption factor	Induces osteopontin expression	Mechanical stress induced PDLCs	[62]
10.	P_2Y_4 P_2Y_6	Remodeling factor	Induces ERK phosphorylation	Stress induced PDLCs	[63]
11.		Behavior of PDLCs	Regulates function and behavior of PDLCs through ATP-connexin 43 channels	Continuous compressive stress induced PDLCs	[11]
12.		Pain factor	Control nociceptive pain due to orthodontic tooth movement	Mechanical stimulated PDLCs	[64]

Roles of eATP in various functions of PDLCs

eATP has numerous effects on the functions of PDLCs. It depends on different receptor interactions. One or more subtypes of purinergic P_2 receptors are found on all types of cells. Previous study's result clarified P_2X_7, P_2Y_1, P_2Y_2, P_2Y_4, P_2Y_6 and P_2Y_{12} receptors expression has been detected in the periodontal ligament cells, that was detected in the 9 days cultured conditions [53]. As ATP can bind to various P_2 receptors, the interaction of eATP and P_2 receptor signalling had different functions on PDLCs, including proliferative function, inflammatory response, immunosuppressive function, osteogenesis, bone destructive function, and other various functions. One receptor can involve in different mechanism of PDLCs' functions, sometimes more than one receptor is involved in each function. Different roles of eATP affect the numerous functions of PDLCs through different signalling, as shown in Table 3.

Effects of eATP on the proliferation of PDLCs

eATP modulates the proliferation of different cell types through specific purinergic receptors. Extracellular ATP and slowly hydrolyzable ATP (ATPγS) suppress the PDLCs proliferation but not the same mechanism. ATP induced PDLCs growth arrest by increasing $p21^{WAF1/cip1}$ that regulates cell proliferation by inhibiting the cell cycle through the cyclin kinase pathway. Extracellular ATPγS induced cellular apoptotic responses. Ectonucleotidases including CD39 which are present in serum rescued the suppressive effect of PDLCs proliferation by ATP and ATPγS [54].

Effects of eATP on the inflammatory function of PDLCs

Mechanical stress induced the release of ATP by PDLCs. The released ATP activates specific purinergic P_2 receptors on the cell surface and has been shown to regulate the trigger of pro-inflammatory cytokines/ chemokines. ATP induces the maturation or the release of pro-inflammatory cytokines /chemokines by PDLSCs. P_2X_7 receptor agonist (BzATP) enhanced the release of IL8 and CCL20 without influencing cell viability. Specific P_2X_7 receptor irreversible inhibitor, oxidized ATP (oATP) or A-74003 counteracted the eATP-induced IL8 and CCL 20 release. This inductive effect is followed by an increase in intracellular Ca^{2+} signalling. Generally, these results suggested that mechanical stress induced pro-inflammatory chemokines IL8 and CCL20 release by PDLSCs through eATP-P_2X_7 receptor interaction [55].

Mechanical stress is also involved in the maintenance of periodontium homeostasis by controlling the major pro-inflammatory mediator IL1β processing and release by PDLCs. Continuous compressive loading upregulated IL1β expression through the release of ATP in PDLCs. IL1β expression was markedly inhibited by a P_2X_7 receptor inhibitor or siRNA targeting the P_2X_7 receptor. As the P_2X_7 receptor is an ion channel receptor mostly permeable to calcium, intracellular calcium inhibitors markedly inhibited eATP-induced IL1β expression. According to this result, eATP-P_2X_7 receptor signalling and intracellular calcium signalling mechanisms are importantly imparted in mechanical stress-induced PDLCs inflammation through induction of pro-inflammatory cytokine, IL1 β production [12]. In next to the latter study, the role of pannexin1 (Panx1) in ATP-induced IL1 β expression in PDLCs was examined. The release of ATP is decreased by using a Panx1 inhibitor. Blocking Panx 1 also inhibited the release of IL1β which was induced by mechanical stress or ATP. Vesicular trafficking inhibitors reduced the release of IL1β by stimulated cells. Therefore, Panx-1 is contributed to the release of ATP and also to the release of IL1β induced by mechanical stress or ATP treatment [56].

Effects of eATP and immunomodulatory function of PDLCs

The immunomodulatory function of PDLCs is very important in host immune responses by suppressing inflammation, initiating the repairing process, and getting efficient regeneration. eATP

stimulates the immunomodulatory function of PDLCs by promoting immunomodulatory molecules IDO and IFNγ release. Inhibition of P_2X_7 receptor by using chemical P_2X_7 antagonists; BBG and KN62, siRNA targeting P_2X_7 receptor, calcium chelator (EGTA), and PKC inhibitor significantly reduced eATP-induced IDO and IFNγ expression. Specific P_2X_7 receptor agonists (BzATP) dramatically induced eATP induced these two molecules' expression. Hence P_2X_7 receptor activation and intracellular calcium signalling are related to an immunomodulatory property of PDLCs. The eATP takes part in the immunosuppressive action of PDLCs [57].

Effects of eATP on osteogenic differentiation of PDLCs
One of the major functions of PDLCs is the differentiation function. eATP has also a key role in the maintenance of osteogenic differentiation of PDLCs. ATP-P_2X_7 receptor interaction decreases osteogenesis on inflammatory mediated PDLSCs through the PI3k-Akt-mTOR signalling pathway [58]. ATP enhances the osteogenic potential of PDLSCs by enhancing osteogenic genes Runx2 and OCN expression after 1 week of ATP treatment in an osteogenic medium. ATP treatment also demonstrated a highly expressed P_2X_7 receptor in PDLSCs. Moreover, ATP activates the P_2X_7 receptor, enhancing the PDLSCs osteogenesis [59]. PDLCs can differentiate into osteoblastic cells under cyclic tensile force. Continuous cyclic tensile force applied for 6 h stimulated osteogenic protein BMP9 synthesis and induced mineralization of PDLCs within 14 days of mineralization. Loss of function and overexpression experiments using suramin (a broad-spectrum P_2Y antagonist), specific P_2Y_1 antagonist (MRS2179), or specific P_2Y_1 receptor agonist revealed the involvement of P_2Y_1 receptor in the induction of BMP9 synthesis. Experiments using U-73122 (a phospholipase C [PLC] inhibitor), and thapsigargin (enhancer of intracytosolic calcium) also suggested the synthesis of BMP9 is related to an increased level of intracellular Ca^{2+} through the PLC pathway. These results indicated that eATP-P_2Y_1 signalling participated in CTF-induced BMP9 synthesis and in vitro mineralization [60]. Compressive including intermittent compressive force (ICF) and continuous compressive force (CCF) significantly increased extracellular ATP levels and ICF involved in the upregulation of osteogenic gene osterix expression through transforming growth factor β pathway. However, exogenous ATP treatment did not show an effect on the osteogenic differentiation of PDLCs [10].

Effects of eATP on the bone-destructive function of PDLCs
Mechanical stress induced the release of ATP by PDLCS and also promotes osteopontin (OPN) expression in PDLCs via the Rho kinase pathway. Osteopontin is the protein involved in bone destruction. Mechanical stress-induced ATP upregulates OPN which is mediated by the P_2Y_1-Rho kinase signalling pathway. Therefore, stress-induced ATP plays part in alveolar bone destruction [61]. In another study, mechanical stress-induced ATP increased bone-destructive protein RANKL expression. Upregulation of RANKL expression was mediated by the same P_2Y_1 receptor activation but through a different pathway. Indomethacin (an inhibitor of COX), H89 (cAMP-dependent protein kinase inhibitor) and pyrrolidine dithiocarbamate (NFκB inhibitor) inhibited RANKL expression, PGE2 production and NFκB translocation. Thus, eATP participates in the maintenance of bone homeostasis mediated by the P_2Y_1-NFκB-COX-RANKL axis in the periodontal tissue [62]. Therefore, eATP is related to bone homeostasis function by inducing different bone-destructive protein expressions through different signalling pathways.

Effects of eATP on PDL repair
PDLCs are mechanosensitive cells, receiving mechanical stress from dental occlusion or orthodontic tooth movement. Mechanical stress like centrifuge-mediated gravity loading increased ATP in the extracellular environment and extracellular signal-regulated kinases (ERK) phosphorylation in PDLCs. ERK phosphorylation imparts in the remodelling of periodontal tissues. Gravity loading induced ATP release and ERK phosphorylation in PDLCs which in turn would enhance the growth and survival of PDLCs. Stress-induced-ATP is involved in the stimulation of periodontal tissue remodelling via the P_2Y receptor especially P_2Y_4 and P_2Y_6 during orthodontic tooth movement [63].

Effects of eATP on other functions of PDLCs
Continuous compressive stress causes the induction of ATP release by PDLCs. The mechanism of ATP release is dependent on the opening of hemichannel protein especially connexin 43. Also, this mechanism is regulated by the intracellular Ca^{2+} signalling pathway. Nevertheless, hemichannel gap junction proteins play important role in the function and behaviour of the PDLCs [11].

PDLCs respond to orthodontic tooth movement-related nociceptive pain by releasing ATP. Vesicular nucleotide transporter (VNUT) takes part in the uptake of ATP into secretory vesicles and this ATP binds to P_2X_3 receptor on trigerminal nerve resulting in tooth movement-induced pain. VNUT inhibitors (clodronic acid) suppressed the release of ATP induced by mechanical stimulation. Systemic administration of clodronic acid inhibited face-grooming behaviour (an indicator of nociception) followed by 1 day of experimental tooth movement. Moreover, ATP could regulate nociceptive pain control related to orthodontic tooth movement [64].

DISCUSSION AND CONCLUSION
Many previous studies proved that eATP has a variety of effects on the functions of different cell types. ATP acts as an intracellular source of energy as well as involved in various intracellular signalling events. Different cell types including PDLCs could release ATP into the extracellular environment in response to mechanical stimuli and inflammatory conditions, and the released ATP has a possibility to take part in different PDLCs' functions. The released ATP by PDLCs acts as a danger signal that stimulates inflammatory reactions, interestingly it also involves in the immunosuppressive action of PDLCs suggesting its biphasic effects on the bone remodeling of PDLCs. Nevertheless, PDLCs are one of the key players in the homeostasis of the periodontium and PDLCs have various kinds of functions. The role of eATP on PDLCs' functions and the detailed mechanism is lesser compared to other cell types, so many further studies are required to assess the effect of eATP on different PDLCs' functions such as angiogenesis, differentiation, and their detailed mechanism that help to get future successful periodontal regeneration therapy.

The periodontal regeneration is a complicated process, and the final goal of periodontal regeneration is the removal of destructive tissue as well as the replacement of new functional structure. To fulfill this goal, PDLCs therapy is a priority for the regeneration but there are a lot of limitations; for example, inflammatory resident tissues that release inflammatory cytokines and change the regenerative ability of host tissue leading to poor prognosis of the cell treatment and failure. Therefore, adjunct strategies such as different growth factors, natural biomaterials are needed to induce cell homing, promote resident cell proliferation and differentiation, induce immunomodulation of host system, regulate cell signalling to get endogenous periodontal regeneration [65]. Nowadays, many studies found that different growth factors, signalling molecules, drugs used as adjuncts to conventional periodontal therapy. For example, local delivery of recombinant PDGF-BB using βTCP carrier promote periodontal wound healing by inducing the expression of ICTP, VEGF, PDGF [66]. Local application of recombinant FGF to infrabony defect improve alveolar bone growth [67]. Many growth factors and small molecules released by cells become target to improve
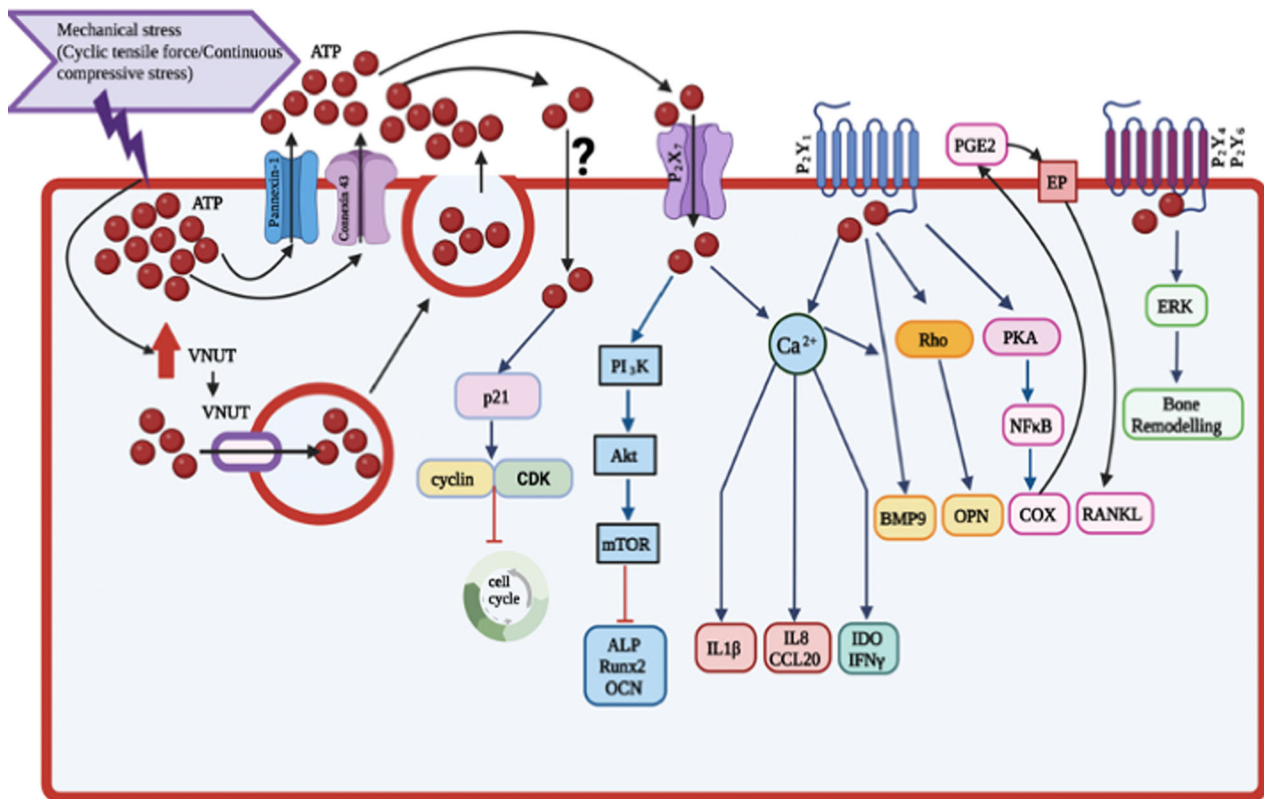
Fig. 2 Effects of adenosine triphosphate on the functions of periodontal ligament cells. This figure summarized the effects of eATP-different P_2 receptor signalling pathway in mechanically stimulated PDLCs (Created with BioRender.com).

regeneration process. According to the previous studies' results, eATP may be a promising therapeutic tool for future periodontal regenerative therapy. The inductive and inhibition effect of eATP via different purinergic P_2 receptor signaling may be applied in creating therapeutic material used as adjuncts for conventional periodontal therapy.

Taken together, eATP plays important role in the control of pro-inflammatory cytokine and chemokine release, inhibition of proliferation, stimulating immunosuppressive action as well as inhibiting or stimulating osteogenic differentiation of PDLCs through various purinergic P_2 receptors and signalling pathways (Fig. 2). These findings improve the knowledge about the released nucleotide ATP support PDLCs to regulate periodontal tissue homeostasis and regeneration process. Understanding the role of eATP on PDLCs functions beneficially applied for the development of new adjunct strategies for the periodontal healing process. With the addition of new advancing technologies, eATP may be utilized as a therapeutic molecule to improve future periodontal regeneration therapy as an adjunct molecule after periodontal surgery to improve the healing process of periodontal defect or used after scaling to control the progress of the periodontal disease. However, further studies are needed to extend the insight of eATP on the periodontal regeneration process.

REFERENCES

1. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. J Periodontal Res. 1991;26:144–54.
2. Liu J, Zhao Z, Ruan J, Weir MD, Ma T, Ren K, et al. Stem cells in the periodontal ligament differentiated into osteogenic, fibrogenic and cementogenic lineages for the regeneration of the periodontal complex. J Dent. 2020;92:103259.
3. McCulloch C, Barghava U, Melcher A. Cell death and the regulation of populations of cells in the periodontal ligament. Cell Tissue Res. 1989;255:129–38.
4. Liu Y, Zheng Y, Ding G, Fang D, Zhang C, Bartold PM, et al. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells. 2008;26:1065–73.
5. Park J-Y, Jeon SH, Choung P-H. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. Cell Transplant. 2011;20:271–86.
6. Lee J-H, Um S, Jang J-H, Seo BM. Effects of VEGF and FGF-2 on proliferation and differentiation of human periodontal ligament stem cells. Cell Tissue Res. 2012;348:475–84.
7. Agarwal S, Chandra CS, Piesco NP, Langkamp HH, Bowen L, Baran C. Regulation of periodontal ligament cell functions by interleukin-1β. Infect Immun. 1998;66:932–7.
8. Issaranggun Na Ayuthaya B, Satravaha P, Pavasant P. Interleukin-12 modulates the immunomodulatory properties of human periodontal ligament cells. J Periodontal Res. 2017;52:546–55.
9. Ryan L, Rachow J, McCarty D. Synovial fluid ATP: a potential substrate for the production of inorganic pyrophosphate. J Rheumatol. 1991;18:716–20.
10. Manokawinchoke J, Pavasant P, Sawangmake C, Limjeerajarus N, Limjeerajarus CN, Egusa H, et al. Intermittent compressive force promotes osteogenic differentiation in human periodontal ligament cells by regulating the transforming growth factor-β pathway. Cell Death Dis. 2019;10:1–21.
11. Luckprom P, Kanjanamekanant K, Pavasant P. Role of connexin43 hemichannels in mechanical stress-induced ATP release in human periodontal ligament cells. J Periodontal Res. 2011;46:607–15.
12. Kanjanamekanant K, Luckprom P, Pavasant P. Mechanical stress-induced interleukin-1beta expression through adenosine triphosphate/P_2X_7 receptor activation in human periodontal ligament cells. J. Periodontal Res. 2013;48:169–76.
13. Heo JS, Han HJ. ATP stimulates mouse embryonic stem cell proliferation via protein kinase C, phosphatidylinositol 3-kinase/Akt, and mitogen-activated protein kinase signaling pathways. Stem Cells. 2006;24:2637–48.
14. Sanz JM, Di, Virgilio F. Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. J. Immunol. 2000;164:4893–8.
15. Caraccio N, Monzani F, Santini E, Cuccato S, Ferrari D, Callegari MG, et al. Extracellular adenosine 5′-triphosphate modulates interleukin-6 production by human thyrocytes through functional purinergic P_2 receptors. Endocrinology. 2005;146:3172–8.
16. Lotfi R, Steppe L, Hang R, Rojewski M, Massold M, Jahrsdörfer B, et al. ATP promotes immunosuppressive capacities of mesenchymal stromal cells by

enhancing the expression of indoleamine dioxygenase. Immun Inflamm Dis. 2018;6:448–55.

17. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontology 2000. 2006;40:164–72.

18. Suwittayarak R, Klincumhom N, Ngaokrajang U, Namangkalakul W, Ferreira JN, Pavasant P, et al. Shear stress enhances the paracrine-mediated immunoregulatory function of human periodontal ligament stem cells via the ERK signalling pathway. Int J Mol Sci. 2022;23:7119.

19. Zhou Y, Hutmacher DW, Sae-Lim V, Zhou Z, Woodruff M, Lim TM. Osteogenic and adipogenic induction potential of human periodontal cells. J Periodontol. 2008;79:525–34.

20. Sawangmake C, Nowwarote N, Pavasant P, Chansiripornchai P, Osathanon T. A feasibility study of an in vitro differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. Biochem Biophys Res Commun. 2014;452:581–7.

21. Sawangmake C, Pavasant P, Chansiripornchai P, Osathanon T. High glucose condition suppresses neurosphere formation by human periodontal ligament-derived mesenchymal stem cells. J Cell Biochem. 2014;115:928–39.

22. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37:1528–42.

23. Fawzy El-Sayed KM, Elahmady M, Adawi Z, Aboushadi N, Elnaggar A, Eid M, et al. The periodontal stem/progenitor cell inflammatory-regenerative cross talk: a new perspective. J Periodontal Res. 2019;54:81–94.

24. Sculean A, Chapple IL, Giannobile WV. Wound models for periodontal and bone regeneration: the role of biologic research. Periodontology 2000. 2015;68:7–20.

25. Seo B-M, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364:149–55.

26. Chiang B, Essick E, Ehringer W, Murphree S, Hauck MA, Li M, et al. Enhancing skin wound healing by direct delivery of intracellular adenosine triphosphate. Am. J. Surg. 2007;193:213–8.

27. Mo Y, Sarojini H, Wan R, Zhang Q, Wang J, Eichenberger S, et al. Intracellular ATP delivery causes rapid tissue regeneration via upregulation of cytokines, chemokines, and stem cells. Front Pharmacol. 2020;10:1502.

28. Howard JD, Sarojini H, Wan R, Chien S. Rapid granulation tissue regeneration by intracellular ATP delivery-a comparison with regranex. PloS One. 2014;9:e91787.

29. Dutta AK, Sabirov RZ, Uramoto H, Okada Y. Role of ATP-conductive anion channel in ATP release from neonatal rat cardiomyocytes in ischaemic or hypoxic conditions. J Physiol. 2004;559:799–812.

30. Robertson J, Lang S, Lambert PA, Martin PE. Peptidoglycan derived from Staphylococcus epidermidis induces Connexin43 hemichannel activity with consequences on the innate immune response in endothelial cells. Biochem J. 2010;432:133–43.

31. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. Nature. 2014;509:310–7.

32. Jacob F, Novo CP, Bachert C, Van Crombruggen K. Purinergic signaling in inflammatory cells: P_2 receptor expression, functional effects, and modulation of inflammatory responses. Purinergic Signal. 2013;9:285–306.

33. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol. 2007;81:1–5.

34. Jiang LH, Hao Y, Mousawi F, Peng H, Yang X. Expression of P_2 purinergic receptors in mesenchymal stem cells and their roles in extracellular nucleotide regulation of cell functions. J Cell Physiol. 2017;232:287–97.

35. Burnstock G. Purine and purinergic receptors. Brain Neurosci Adv. 2018;2:2398212818817494.

36. Junger WG. Immune cell regulation by autocrine purinergic signalling. Nat Rev Immunol. 2011;11:201–12.

37. Burnstock G. Purine and pyrimidine receptors. Cell Mol Life Sci. 2007;64:1471–83.

38. Semenova S, Shatrova A, Vassilieva I, Shamatova M, Pugovkina N, Negulyaev Y. Adenosine-5′-triphosphate suppresses proliferation and migration capacity of human endometrial stem cells. J Cell Mol Med. 2020;24:4580–4.

39. Coppi E, Pugliese AM, Urbani S, Melani A, Cerbai E, Mazzanti B, et al. ATP modulates cell proliferation and elicits two different electrophysiological responses in human mesenchymal stem cells. Stem Cells. 2007;25:1840–9.

40. Seo DR, Kim SY, Kim KY, Lee HG, Moon JH, Lee JS, et al. Cross talk between P_2 purinergic receptors modulates extracellular ATP-mediated interleukin-10 production in rat microglial cells. Exp Mol Med. 2008;40:19–26.

41. Swennen EL, Bast A, Dagnelie PC. Purinergic receptors involved in the immunomodulatory effects of ATP in human blood. Biochem Biophys Res Commun. 2006;348:1194–9.

42. Hide I, Tanaka M, Inoue A, Nakajima K, Kohsaka S, Inoue K, et al. Extracellular ATP triggers tumor necrosis factor-α release from rat microglia. J. Neurochem. 2000;75:965–72.

43. Yilmaz Ö, Sater AA, Yao L, Koutouzis T, Pettengill M, Ojcius DM. ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by Porphyromonas gingivalis. Cell Microbiol. 2010;12:188–98.

44. Solini A, Chiozzi P, Morelli A, Fellin R, Di, Virgilio F. Human primary fibroblasts in vitro express a purinergic P_2X_7 receptor coupled to ion fluxes, microvesicle formation and IL-6 release. J Cell Sci. 1999;112:297–305.

45. Kim H, Walsh MC, Takegahara N, Middleton SA, Shin H-I, Kim J, et al. The purinergic receptor P_2X_5 regulates inflammasome activity and hyper-multinucleation of murine osteoclasts. Sci Rep. 2017;7:1–11.

46. Bilbao PS, Boland R, Santillán G. ATP modulates transcription factors through P_2Y_2 and P_2Y_4 receptors via PKC/MAPKs and PKC/Src pathways in MCF-7 cells. Arch Biochem Biophys. 2010;494:7–14.

47. Nemoto E, Gotoh K, Tsuchiya M, Sakisaka Y, Shimauchi H. Extracellular ATP inhibits IL-1-induced MMP-1 expression through the action of CD39/nucleotidase triphosphate dephosphorylase-1 on human gingival fibroblasts. Int Immunopharmacol. 2013;17:513–8.

48. Davenport AJ, Neagoe I, Bräuer N, Koch M, Rotgeri A, Nagel J, et al. Eliapixant is a selective P_2X_3 receptor antagonist for the treatment of disorders associated with hypersensitive nerve fibers. Sci Rep. 2021;11:1–13.

49. Trabanelli S, Očadlíková D, Gulinelli S, Curti A, Salvestrini V, de Paula Vieira R, et al. Extracellular ATP exerts opposite effects on activated and regulatory $CD4^{++}$ T cells via purinergic P_2 receptor activation. J Immunol. 2012;189:1303–10.

50. Marteau F, Gonzalez NS, Communi D, Goldman M, Boeynaems J-M, Communi D. Thrombospondin-1 and indoleamine 2, 3-dioxygenase are major targets of extracellular ATP in human dendritic cells. Blood. 2005;106:3860–6.

51. Ring S, Enk AH, Mahnke K. ATP activates regulatory T Cells in vivo during contact hypersensitivity reactions. J Immunol. 2010;184:3408–16.

52. Li S, Chen X, Li J, Li X, Zhang T, Hao G, et al. Extracellular ATP is a potent signaling molecule in the activation of the Japanese flounder (Paralichthys olivaceus) innate immune responses. Innate Immun. 2020;26:413–23.

53. Pavasant P, Yongchaitrakul T. Role of mechanical stress on the function of periodontal ligament cells. Periodontology 2000. 2011;56:154–65.

54. Kawase T, Okuda K, Yoshie H. Extracellular ATP and ATPγS suppress the proliferation of human periodontal ligament cells by different mechanisms. J Periodontol. 2007;78:748–56.

55. Trubiani O, Horenstein AL, Caciagli F, Caputi S, Malavasi F, Ballerini P. Expression of P_2X_7 ATP receptor mediating the IL8 and CCL20 release in human periodontal ligament stem cells. J Cell Biochem. 2014;115:1138–46.

56. Kanjanamekanant K, Luckprom P, Pavasant P. P_2X_7 receptor–P annexin1 interaction mediates stress-induced interleukin-1 beta expression in human periodontal ligament cells. J Periodontal Res. 2014;49:595–602.

57. Kyawsoewin M, Limraksasin P, Ngaokrajang U, Pavasant P, Osathanon T. Extracellular adenosine triphosphate induces IDO and IFNγ expression of human periodontal ligament cells through P_2X_7 receptor signaling. J Periodontal Res. 2022;57:742–53.

58. Xu X-Y, He X-T, Wang J, Li X, Xia Y, Tan Y-Z, et al. Role of the P_2X_7 receptor in inflammation-mediated changes in the osteogenesis of periodontal ligament stem cells. Cell Death Dis. 2019;10:1–17.

59. Wen-jing L, Hui-zhen C, Zhi-yong Z. Expression level and effect of P_2X_7 receptor mRNA during osteogenic differentiation of human periodontal ligament stem cells. Shanghai J Stomatol. 2020;29:365.

60. Tantilertanant Y, Niyompanich J, Everts V, Supaphol P, Pavasant P, Sanchavanakit N. Cyclic tensile force stimulates BMP9 synthesis and in vitro mineralization by human periodontal ligament cells. J Cell Physiol. 2019;234:4528–39.

61. Wongkhantee S, Yongchaitrakul T, Pavasant P. Mechanical stress induces osteopontin via ATP/P_2Y_1 in periodontal cells. J Dent Res. 2008;87:564–8.

62. Luckprom P, Wongkhantee S, Yongchaitrakul T, Pavasant P. Adenosine triphosphate stimulates RANKL expression through P_2Y_1 receptor–cyclo-oxygenase-dependent pathway in human periodontal ligament cells. J Periodontal Res. 2010;45:404–11.

63. Ito M, Arakawa T, Okayama M, Shitara A, Mizoguchi I, Takuma T. Gravity loading induces adenosine triphosphate release and phosphorylation of extracellular signal-regulated kinases in human periodontal ligament cells. J Investigative Clin Dent. 2014;5:266–74.

64. Mizuhara M, Kometani-Gunjigake K, Nakao-Kuroishi K, Toyono T, Hitomi S, Morii A, et al. Vesicular nucleotide transporter mediates adenosine triphosphate release in compressed human periodontal ligament fibroblast cells and participates in tooth movement-induced nociception in rats. Arch Oral Biol. 2020;110:104607.

65. Yin Y, Li X, He X, Wu R, Sun H, Chen F. Leveraging stem cell homing for therapeutic regeneration. J Dent Res. 2017;96:601–9.

66. Cooke JW, Sarment DP, Whitesman LA, Miller SE, Jin Q, Lynch SE, et al. Effect of rhPDGF-BB delivery on mediators of periodontal wound repair. Tissue Eng. 2006;12:1441–50.

67. Kitamura M, Akamatsu M, Kawanami M, Furuichi Y, Fujii T, Mori M, et al. Randomized placebo-controlled and controlled non-inferiority phase III trials comparing trafermin, a recombinant human fibroblast growth factor 2, and enamel matrix derivative in periodontal regeneration in intrabony defects. J Bone Miner Res. 2016;31:806–14.

68. Park J-Y, Park CH, Yi T, Kim S-N, Iwata T, Yun J-H. rhBMP-2 pre-treated human periodontal ligament stem cell sheets regenerate a mineralized layer mimicking dental cementum. Int J Mol Sci. 2020;21:3767.

69. Dennison DK, Vallone DR, Pinero GJ, Rittman B, Caffesse RG. Differential effect of TGF-β1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. J Periodontol. 1994;65:641–8.

70. Iwasaki K, Komaki M, Mimori K, Leon E, Izumi Y, Ishikawa I. IL-6 induces osteoblastic differentiation of periodontal ligament cells. J Dent Res. 2008;87:937–42.

71. Chaikeawkaew D, Everts V, Pavasant P. TLR3 activation modulates immunomodulatory properties of human periodontal ligament cells. J Periodontol. 2020;91:1225–36.

72. Klincumhom N, Chaikeawkaew D, Adulheem S, Pavasant P. Activation of TLR3 enhance stemness and immunomodulatory properties of periodontal ligament stem cells (PDLSCs). Interface Oral Health Sci 2016. 2016;205–16.

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ADDITIONAL INFORMATION

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APPENDIX

Appendix 1 List of abbreviations

eATP	Extracellular adenosine triphosphate
ATP	Adenosine triphosphate
PDLCs	Periodontal ligament cells
VEGF	Vascular Endothelial Growth Factor
FGF2	Fibroblast growth factor 2
IL	Interleukin
PKC	Protein kinase C
PI_3K	Phosphatidylinositol 3-kinase
Akt	Ak mouse strain transforming serine-threonine protein kinase
MAPK	Mitogen activated protein kinase
MG-5	Microglial cell line 5
IDO	Indoleamine-pyrrole 2,3-dioxygenase

Table a. continued

BMSCs	Bone marrow mesenchymal stem cells
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta Analyses
PDL	Periodontal ligament
PDLSCs	Periodontal ligament stem cells
rhBMP-2	Recombinant bone morphogenetic protein-2
PDGF	Platelet-derived growth factor
TGF-β1	Transforming growth factor β1
LPS	Lipopolysaccharides
IFNγ	Interferon-gamma
HLA-G	Human leukocyte antigen G
Poly I:C	polyinosinic-polycytidylic acid
TLR3	Toll like receptor 3
iATP	Intracellular adenosine triphosphate
TNFα	Tumor necrosis factor alpha
Mg	Magnesium
DAMP	Danger-Associated- Molecular-Pattern Molecule
P_2	Purinergic 2 receptor
P_2YRs	P_2Y family of purinergic receptors
P_2YRs	P_2X family of purinergic receptors
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
UDP	Uridine diphosphate
UTP	Uridine triphosphate
NAD	Nicotinamide-adenine dinucleotide
PLC	Phospholipase C
IP_3	Inositol triphosphate
AC	Adenylyl cyclase
cAMP	Cyclic adenosine monophosphate
K^+	Potassium
Ca^{2+}	Calcium
PKA	Protein kinase A
NALP3	NACHT, LRR and PYD domains-containing protein 3
P. gingivalis	*Porphyromonas gingivalis*
ERK	Extracellular signal-regulated kinases
p38	38-kDa protein
JNK	c-Jun N-terminal kinase
MCF-7	Breast cancer cell line acronym for Michigan Cancer Foundation-7
MMPs	Matrix metalloproteinases
CD	Cluster of differentiation
CD39	Ectonucleoside triphosphate diphosphohydrolase-1

Table a. continued

CD^{4+} T cells	Helper T cells
Treg	Regulatory T cells
ATPγS	Slowly hydrolysable ATP
BzATP	2'(3')-*O*-(4-Benzoylbenzoyl) adenosine-5'-triphosphate tri(-triethylammonium) salt
CCL20	Chemokine (C-C motif) ligand 20
oATP	Oxidized adenosine triphosphate
A-74003	Artificial P$_2$X$_7$ receptor antagonist
siRNA	Small interfering RNA
Panx1	Pannaxin-1
BBG	Brilliant Blue G
KN62	4-[(2 S)-2-(N-Methylisoquinoline-5-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl) propyl] phenyl isoquinoline-5-sulfonate
EGTA	Ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid
mTOR	Mammalian target of rapamycin
BMP9	Bone morphogenetic protein 9
MRS2179	Competitive antagonist at P$_2$Y$_1$ receptors
U-73122	Phospholipase C and 5-lipooxygenase inhibitor
CTF	Cyclic tensile force
ICF	Intermittent compressive force
CCF	Continuous compressive force

Table a. continued

OPN	Osteopontin
Rho	Ras homologous (Rho) protein
RANKL	Receptor activator of nuclear factor-kappa B ligand
COX	Cyclo-oxygenase
H-89	Potent and selective inhibitor of cyclic AMP-dependent protein kinase
NFκB	Nuclear factor kappa B
PGE2	Prostaglandin E2
VNUT	Vesicular nucleotide transporter
Runx2	Runt related transcription factor 2
OCN	Osteocalcin
βTCP	Beta tricalcium phosphate
ICTP	pyridinoline cross- linked carboxyterminal telopeptide of Type I collagen