Perspective

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Mechanical force modulates inflammation and immunomodulation in periodontal ligament cells

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Abstract: Mechanical forces control a multitude of biological responses in various cells and tissues. The periodontal ligament, located between the tooth's root and alveolar bone, is a major tissue compartment that is incessantly subjected to such mechanical stimulation through either normal or abnormal oral functionality. It is now known that mechanical stimulation activates periodontal ligament stem cells (PDLSCs) to modulate periodontal immunity and regulate inflammation – a basic feature of periodontal disease that affects virtually every human during their lifetime. For instance, shear stress induces the expression of immunomodulatory-related gene, indoleamine 2,3-dioxygenase (IDO). IDO cleaves l-tryptophan, resulting in increased l-kynurenine levels that, in turn, further promote regulatory T-cell differentiation and inhibit T cell proliferation. These and other related data reinforce the notion that mechanical stimulation plays a crucial role in controlling inflammation and immunomodulation of periodontal tissues. Further investigations, however, are warranted to evaluate the immunomodulatory features of PDLSCs so as to understand the pathological basis of periodontal disease and translate these into clinical interventions.

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Introduction

In general, mechanical forces exerted on tissues or cells (mechanostimulation) are pivotal in regulating tissue regeneration, destruction, or controlling their cellular behavior [1]. In the oral cavity, excessive forces exerted due to chewing and occlusion, for instance, can be detrimental to the tooth-bearing periodontal tissues. Conversely, the application of optimal force to periodontal structures yields desired treatment outcomes, as seen in orthodontic tooth movement. Through mechanosensing, cells can convert external mechanical stimuli into biochemical signaling pathways, triggering intracellular responses that enable them to adapt to diverse environmental conditions [2]. The interpretation of mechanical forces by cells is a multifaceted process influenced by a complex interplay between various factors. These factors include cellular signaling pathways, extracellular fluid properties, tissue mechanics, fluid flow patterns, and compressive forces.

Periodontal Ligament Stem Cells (PDLSCs) are particularly important players in this process. Acting as mechanosensors, they translate these forces into biochemical signals that not only regulate their own behavior but also influence surrounding immune cells. PDLSCs can respond to different types of forces by expressing various immunomodulatory molecules, creating a finely tuned environment that promotes periodontal health and tissue repair.

Extensive efforts have been made to understand these fundamental mechanisms. Research has placed particular emphasis on elucidating the role of integrin signaling and ion channel modulation in mechanosensing by PDLSCs. Integrins are transmembrane linkers mediating the interaction between the cytoskeleton of cells and the extracellular matrix that permits cells to grip their surroundings [3]. Integrin activation leads to the triggering of intracellular signaling and remodeling of the cytoskeleton. Mechanoreceptors, such as

those in the Piezo and TRP families, allow ions to influx to the cytoplasm upon receiving mechanical stimuli, further activating intracellular signaling pathways.

These mechanical cues are translated by PDLSCs into biological responses that modulate periodontal immunity. Acting as mechanosensors, PDLSCs are equipped with integrins and mechanoreceptors that detect and transduce forces into intracellular signals. These signals can activate various pathways, influencing the production of immunomodulatory molecules by PDLSCs. For instance, PDLSCs can express factors like interleukin-10 (IL-10) with anti-inflammatory properties in response to optimal forces, promoting a healthy periodontal environment. Conversely, excessive mechanical stress might trigger the release of pro-inflammatory cytokines. By fine-tuning the immune response, PDLSCs play a critical role in maintaining a balanced and healthy periodontal environment.

The periodontal ligament is a dense connective tissue that firmly secures the tooth within its alveolar socket. This vital tissue endures constant exposure to diverse mechanical forces generated by normal oral activities such as mastication, speech, and deglutition. Recent research highlights the remarkable ability of periodontal ligament cells to sense these mechanical stimuli, a process termed mechanosensing [1]. For instance, when teeth lack proper function and experience reduced chewing forces, it can lead to a narrowing of the periodontal space, a misalignment of the fibers that support the periodontal ligament, and changes in the composition of the material surrounding the tooth (extracellular matrix) [2]. This exemplifies the central contribution of mechanical forces in the homeostasis of the periodontal ligament. Residing within the periodontal ligament are PDLSCs of mesenchymal origin, similar to other mesenchymal stem cells (MSCs), which possess multipotent differentiation potential as well as immunomodulatory capacity [4]. However, PDLSCs go a step further by acting as specialized mechanosensors. They are equipped with integrins and mechanoreceptors that detect and translate mechanical forces into biochemical signals. These signals, in turn, activate various pathways within PDLSCs, influencing the production of immunomodulatory molecules.

Indeed, MSCs express or secrete immunomodulatory molecules that either suppress immune cell function or stimulate anti-inflammatory immune cells while partaking in healing and/or regeneration of the tissues [5]. The pivotal molecules that play a role in this immunosuppressive function of MSCs are Indoleamine 2,3-dioxygenase (IDO), transforming growth factor β1 (TGF-β1), and IL-10 [5]. Fully differentiated PDLSCs, being of mesenchymal origin, also possess inherited immunomodulatory functions. When exposed to specific signals, PDLSCs secrete molecules that suppress the immune system's activity. These molecules can influence the behavior of various immune cells, including peripheral blood mononuclear cells (PBMCs). Interestingly, this interaction can also lead to PDLSCs themselves acquiring immunosuppressive properties. This complex process involves the release of both pro-inflammatory and anti-inflammatory molecules, such as adenosine triphosphate (ATP) and TGF-β1 [6].

Immunomodulatory potential of PDLSCs

PDLSCs are immunomodulatory in nature by virtue of a multitude of attributes. This functionality entails either direct cell-to-cell interactions or indirect paracrine mechanisms via secreted molecules. During inflammation, they suppress neutrophil apoptosis, PDLSCs can secrete FLIP, a protein that blocks caspase activation, a critical step in apoptosis for neutrophils. This allows neutrophils to survive longer and continue their phagocytic activity against invading pathogens. While promoting monocyte numbers and differentiation towards M1 pro-inflammatory macrophages, in addition to secreting pro-inflammatory cytokines such as IL-β, TNF-α, IFN-γ. These cytokines bind to receptors on monocytes, promoting their differentiation towards by enhanced phagocytosis, antigen presentation, and release of inflammatory mediators [7]. PDLSCs exert multifaceted immunomodulatory effects. They suppress B cell proliferation, migration, and differentiation while simultaneously mitigating B cell apoptosis through the secretion of IL-6 [8]. They also downregulate CD1b expression on dendritic cells, thereby hindering their maturation [9]. Furthermore, they promote macrophage polarization towards the anti-inflammatory M2 phenotype [10] and inhibit the proliferation of PBMC [11].

Mechanical stimuli act as potent inducers for the expression and upregulation of three key molecules in PDL cells: i) IDO, ii) TGF-β1, and iii) PGE2. IDO acts as a potent immunomodulator by critically regulating T cell function. PDLSCs can upregulate IDO expression, leading to T cell starvation and reduced proliferation. It achieves this through the depleting tryptophan levels, which consequently leads to the induction of T cell apoptosis, reduction of T cell proliferation, and the promotion of Treg differentiation [12]. Depending on the cell type and the microenvironment, TGF-β1 also regulates numerous cellular responses, including the functions of T cells, B cells, mast cells, dendritic cells, and macrophages. Finally, TGF-β1 suppresses T cell proliferation and promotes differentiation of

$CD4^+$ T cells towards immunosuppressive $FOXP3^+$ T cells. PDLSCs can suppress the production of antibodies by B cells, which are essential components of the adaptive immune response. This might be achieved through decreased B cell activation, which PDLSCs may interact with B cells and antigen-presenting cells, hindering their interaction and subsequent B cell activation and cytokine modulation, which in turn might influence the cytokine milieu, decreasing the levels of pro-inflammatory cytokines that stimulate B cell activation [8]. PDLSCs can influence macrophage polarization, favouring the anti-inflammatory M2 phenotype over the pro-inflammatory M1 phenotype. M2 macrophages play a crucial role in tissue repair and debris clearance while promoting immune tolerance. They may enhance the phagocytic activity of macrophages, allowing them to more efficiently clear pathogens and debris from the inflamed site [10].

Multiple signalling pathways converge to regulate the expression of IDO and human leukocyte antigen G (HLA-G) is controlled by IFNγ in human periodontal ligament cells [13]. In an alternative pathway, Toll-like receptor 3 (TLR3) activation in periodontal ligament cells promotes an immunosuppression microenvironment by upregulation of IFNγ, IDO, and HLA-G and suppresses PBMC proliferation and upregulate $FOXP3^+$ T cells, a marker for Treg cell expression [14]. The inflammatory cytokine, such as IL-12, can also enhance the expression of these immunosuppression-associated genes, IFNγ, IDO, and HLA-G [13].

Mechanical forces modulate the immunomodulatory function of PDLSCs

Several studies have demonstrated that mechanical forces regulate specific biological activities of periodontal ligament cells [4, 6, 15]. These activities include cytokine and growth factor secretion and cytoskeleton activation associated with inflammation, cell remodeling and differentiation, and immunomodulation. Cyclic stretch exposure, continuous compressive forces, or intermittent compressive forces can significantly regulate the differential expression of mRNA within periodontal ligaments cell. Excessive mechanical forces can upregulate a cascade of inflammatory and osteoclast-activating factors within the periodontal ligament. Cytokines such as IL-β, TNF-α, and IFN-γ play a pivotal role in this process. These cytokines act as key modulators, refining the immune response to aseptic inflammation triggered by mechanical overload. Furthermore, PDLSCs subjected to mechanical forces can directly influence the immune response through cell-to-cell contact. This interaction triggers B and T lymphocyte differentiation, chemotaxis, and proliferation [7, 8].

As described above, shear stress exposure upregulates *IDO* mRNA expression in PDLSCs, leading to a significantly increased kynurenine level and both latent and active forms of TGF-β1 production via the ERK1/2 pathway [4] (Figure 1). IDO depletes tryptophan, an essential amino acid for T cell activation, thereby suppressing their proliferation. Additionally, mechanical forces can promote the production of total and active TGF-β1 in PDLSCs. A study investigating the effects of mechanical stress on PDLSCs employed a co-culture system. T cells were exposed to a conditioned medium collected from PDLSCs previously subjected to shear stress. This experiment revealed a significant reduction in the proliferation of $CD4^+$ T cells [4]. The same workers observed an increased number of $CD4+CD25^{hi}CD127^{-/low}$ Treg cell populations within the co-culture system, suggesting that PDLSCs exposed to shear stress promote Treg cell differentiation (Figure 1). These findings strongly provide compelling evidence for the hypothesis that PDLSCs exhibit

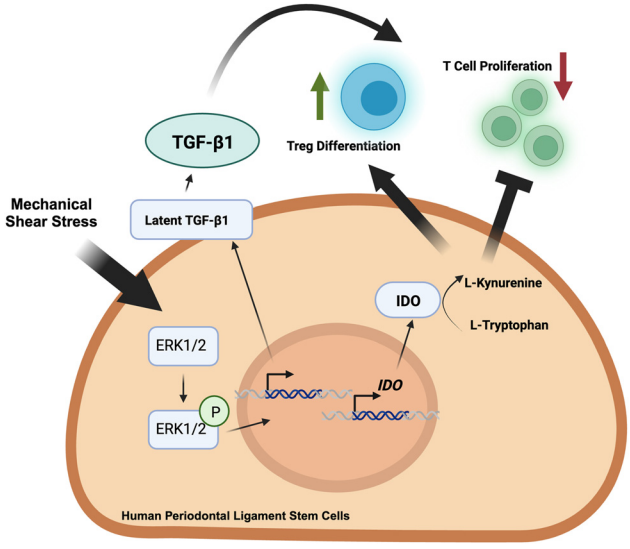

Figure 1: Schematic diagram depicts the mechanism by which shear stress triggers immunosuppressive properties in hPDLSCs via ERK-induced IDO expression. Shear stress activates the ERK signalling pathway, leading to the upregulation of IDO expression. IDO, catabolizes tryptophan, resulting in elevated kynurenine levels within hPDLSCs. Additionally, ERK1/2 activation promotes the production of total and active TGF-β1, which is secreted into the extracellular matrix or conditioned medium. The combined effect of upregulation of kynurenine and TGF-β1 secretion by PDLSCs leads to the suppression of CD4+ T cell proliferation and promotes Treg cell differentiation (modified from Suwittayarak et al. [4] 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. Under Creative Commons Attribution (CC BY) license (5). Created with Biorender.com).

paracrine-mediated immunosuppressive properties when stimulated by mechanical forces.

Mechano-stimulated ATP release and the immunomodulatory function of PDLSCs

The specific effects of ATP-mediated immunomodulation can vary depending on the type and duration of the mechanical force applied. ATP release induced by mechanical force appears to play a significant role in both the inflammation-inducing and immunomodulation properties of periodontal ligament cells, as follows. There is a burst of ATP release after mechanical stimuli of periodontal ligament cells. This occurs via membrane-bound Pannexin 1 and Connexin 43 pathways, leading to ATP exocytosis (Figure 2). Subsequently, the released ATP interacts with P2X7 receptors on the cell membrane, leading to increased intracellular calcium levels and further upregulating the expression of IDO and IFNγ via the protein kinase C (PKC) pathway, both of which contribute to the immunomodulatory functions of these cells [16]. Other signaling pathways besides PKC might also be involved in the mechanotransduction of immunomodulatory responses in PDLSCs. Binding of ATP to the P2Y1 receptor activates the PKA signaling pathway, leading to increased production of PGE2, a pro-inflammatory mediator. Furthermore, genes associated with inflammation and immune modulation are upregulated. The data on the above have been shown in experiments with contrived exposure of periodontal ligament cells further confirming these findings. These studies demonstrate that exposure to ATP can induce the expression of IDO and IFNγ, supporting the role of ATP in immunomodulation [16].

Besides immunomodulation, exogenous ATP also regulates inflammation in periodontal ligament cells through mechanical force-induced inflammatory cytokine expression IL-1β via the ATP pathway [15] (Figure 2). Studies have demonstrated that inhibiting intracellular calcium signalling with specific inhibitors significantly impairs the ability of externally applied ATP to stimulate IL-1β production in periodontal ligament cells [15]. This finding strongly suggests that ATP/P2 purinergic receptors play a crucial role in mechanical force-induced inflammation within periodontal ligament cells. Hence, the mechanical force-induced release of ATP in PDL cells appears to exert a bimodal effect, impacting both the inflammatory and immunomodulatory properties of these cells.

Conclusion and perspectives

Applying varying mechanical forces to periodontal ligament impacts a number of gene expression pathways that modulate the interplay between inflammation and immunomodulation within the periodontium's innate and adaptive immune systems. PDLSCs act as key regulators of this finely tuned and temporally coordinated inflammatory and immunomodulatory response within the periodontal ligament of this tooth-bearing tissue.

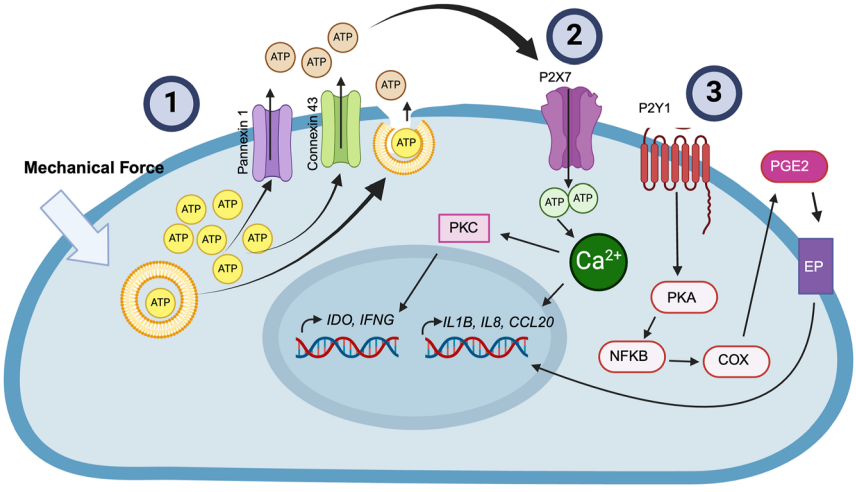

Figure 2: A schematic diagram illustrating the manner in which mechanical forces may impact inflammation and immunomodulation of periodontal ligament cells. (1) Mechanical stimulation induces ATP release from periodontal ligament cells through pathways Pannexin 1 and Connexin 43 hemichannels and exocytosis. (2) Activation of P2X7 receptor by ATP leads to increased intracellular calcium levels and the activation of PKC. This pathway subsequently upregulates the expression of IDO and IFN-γ, both of which contribute to immunomodulation. (3) Binding of ATP to P2Y1 receptor activates the PKA signaling pathway, leading to increased production of PGE2, a well-known pro-inflammatory mediator. This interplay between ATP release and receptor signaling ultimately leads to the upregulation of genes associated with both inflammation and immunomodulation in periodontal ligament cells. Created by Biorender.com.

A variety of inflammatory signaling molecules can selectively induce distinct immunomodulatory responses through specific proteins. For instance, the initial activation of IL-β, TNF-α, and IFN-γ often triggers intricate signaling pathways that convert mechanical stimuli into biochemical signals. However, understanding of the precise molecular mechanisms directly impacting the activation of PDLSCs due to mechanical force, as well as the effect of different force types on these cells, remains limited. It is therefore crucial to investigate the immunomodulatory functions of PDLSCs, particularly because of the varying modalities of mechanical force applications currently seen in various dental management procedures. Therefore, extensive research is warranted to fully comprehend the immunomodulatory capabilities of PDLSCs. This knowledge will not only advance our fundamental understanding of these phenomena but also lead to further insights into how mechanical forces translate into biological outcomes within the periodontal ligament cells.

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References

1. Chukkapalli SS, Lele TP. Periodontal cell mechanotransduction. Open Biol. 2018;8. https://doi.org/10.1098/rsob.180053.
2. Nan DN, Everts V, Ferreira JN, Trachoo V, Osathanon T, Klincumhom N, et al. Alteration of extracellular matrix proteins in atrophic periodontal ligament of hypofunctional rat molars. BDJ Open 2023;9:31.
3. Yang L, Chen H, Yang C, Hu Z, Jiang Z, Meng S, et al. Research progress on the regulatory mechanism of integrin-mediated mechanical stress in cells involved in bone metabolism. J Cell Mol Med 2024;28: e18183.
4. 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. https://doi.org/10.3390/ijms23137119.
5. Wu X, Jiang J, Gu Z, Zhang J, Chen Y, Liu X. Mesenchymal stromal cell therapies: immunomodulatory properties and clinical progress. Stem Cell Res Ther 2020;11:345.
6. 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-beta pathway. Cell Death Dis 2019;10:761.
7. Behm C, Zhao Z, Andrukhov O. Immunomodulatory activities of periodontal ligament stem cells in orthodontic forces-induced inflammatory processes: current views and future perspectives. Front Oral Health 2022;3. https://doi.org/10.3389/froh.2022.877348.
8. Liu O, Xu J, Ding G, Liu D, Fan Z, Zhang C, et al. Periodontal ligament stem cells regulate B lymphocyte function via programmed cell death protein 1. Stem Cell 2013;31:1371–82.
9. Shin C, Kim M, Han JA, Choi B, Hwang D, Do Y, et al. Human periodontal ligament stem cells suppress T-cell proliferation via down-regulation of non-classical major histocompatibility complex-like glycoprotein CD1b on dendritic cells. J Periodontal Res 2017;52:135–46.
10. Liu J, Chen B, Bao J, Zhang Y, Lei L, Yan F. Macrophage polarization in periodontal ligament stem cells enhanced periodontal regeneration. Stem Cell Res Ther 2019;10:320.
11. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S. Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 2009;219:667–76.
12. Prendergast GC, Malachowski WP, DuHadaway JB, Muller AJ. Discovery of Ido1 inhibitors: from bench to bedside. Cancer Res 2017; 77:6795–811.
13. 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.
14. Chaikeawkaew D, Everts V, Pavasant P. TLR3 activation modulates immunomodulatory properties of human periodontal ligament cells. J Periodontol 2020;91:1225–36.
15. Kanjanamekanant K, Luckprom P, Pavasant P. Mechanical stress-induced interleukin-1beta expression through adenosine triphosphate/P2X7 receptor activation in human periodontal ligament cells. J Periodontal Res 2013;48:169–76.
16. Kyawsoewin M, Limraksasin P, Ngaokrajang U, Pavasant P, Osathanon T. Extracellular adenosine triphosphate induces Ido and IFNgamma expression of human periodontal ligament cells through P(2) X(7) receptor signaling. J Periodontal Res 2022;57: 742–53.