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REVIEW ARTICLE

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The Impact of Aspirin on Stem Cells and Growth Factors: Roles in Dentistry

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Abstract

The periodontal ligament (PDL) contains a unique population of mesenchymal stem cells (MSCs), also known as PDL stem cells (PDLSCs). The regenerative properties of PDLSCs offer much potential for stem cell-based therapy, particularly for periodontal or bone regeneration. Aspirin (ASA) is a widely used nonsteroidal anti-inflammatory drug (NSAID) that has been reported to modulate a variety of diseases, such as cardiovascular, diabetes, and cancer. This review article focuses on the impacts of ASA on various stem cells. First, we will explain what constitutes PDLSCs and their derivation from periodontal tissues. Then we will discuss the mechanisms of ASA and its effect on periodontal tissues. Next, we focus on aspirin's effects on the differentiation of various types of stem cells. Finally, we investigate the effects of ASA on growth factors that could enhance the osteoblastic potential of derived stem cells.(**International Journal of Biomedicine. 2023;13(2):188-193.**)

Keywords: acetylsalicylic acid • periodontal ligament • stem cells

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Abbreviations

ASA, acetylsalicylic acid; **ARS**, Alizarin red S; **BMP**, bone morphogenetic protein; **BMMSCs**, bone marrow mesenchymal stem cells; **COX**, cyclooxygenase; **GDF**, growth differentiation factor; **FGF,** fibroblast growth factor; **MSCs**, mesenchymal stem cells; **NSAID**, nonsteroidal anti-inflammatory drug; **PDL**, periodontal ligament; **PDLSCs**, PDL stem cells; **PEG**, poly(ethylene glycol); **PG**, prostaglandin; **qPCR**, quantitative polymerase chain; **SCs**, stem cells; **TSCs**, tendon stem cells; **VEGF**, vascular endothelial growth factor.

Periodontal Ligament Stem Cells (PDSLCs) derived from Periodontal Ligament (PDL) tissue

Periodontal ligament (PDL) is a soft dynamic connective tissue located between the cementum of the root and the alveolar bone.[1,2] PDL tissues consist of heterogeneous populations of cells, including fibroblast, endothelial, osteoblast, and cementoblast cells.[3-6] They play a pivotal role in maintaining homeostasis and regeneration of the periodontal tissues.[7,8] The PDL cells are formed by the cells residing within dental follicle cells during embryogenesis.[9]

The human PDLSCs were first isolated from human-impacted third molars.[10] Numerous studies then indicated that mesenchymal stem cells (MSCs) isolated from PDL have properties that are similar to bone marrow mesenchymal stem cells (BMMSCs).[8,10-12] Like BMMSCs, the PDLSCs demonstrated the ability to self-renew and generate clonogenic adherent colonies with spindle and elongated-shaped cells. Moreover, PDLSCs are capable of forming various types of mesodermal origin cells, such as osteoblasts, chondrocytes, cementoblasts, and neural-like cells.[1,8,10,12]

Periodontal regeneration is a method of regenerative therapy to return the periodontal tissues (including gingiva, root cementum, alveolar bone, and the PDL) to their original healthy condition through the restoration of form and

function of lost structures.[13] NSAIDs are widely used as an analgesic agent in healthcare. They may be used in managing orthopedic patients pre/post-surgery, to address acute or chronic inflammation. However, such use of NSAIDs may have an undesirable impact on stem cell function, particularly in periodontal or bone regeneration.[14-18]

Aspirin and its mechanism

ASA, also known as acetylsalicylic acid, is a NSAID mainly used as an analgesic, antipyretic, and anti-inflammatory. ASA exerts its anti-inflammatory action by suppressing the production of prostanoids (thromboxanes, prostacyclins, and prostaglandins),[19] which are produced by COX-1 and COX-2 enzymes. Prostaglandins (PG), known as prostanoids, are formed when arachidonic acid is released from the plasma membrane by phospholipases and metabolized by the sequential actions of prostaglandin G/H synthase, or COX. COX is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities.[20] PG synthesis is triggered by COX activity. The COX enzyme exists in two different forms, namely COX-1 and COX-2. COX-1 has a "housekeeping" role, is constitutively expressed, and plays important roles in physiological functions, such as platelet aggregation, gastric lining protection, and homeostasis.[21] COX-2 is an inducible enzyme that is implicated in pathophysiological processes, which can lead to inflammation, pain, and fever.[22]

COX-1 is expressed in both normal and fractured bones, while COX-2 is upregulated, especially during the initial stages of the bone repair process. Both osteoblasts and osteoclasts produce prostaglandins, where PGE2 is the most abundant PG synthesized. COX and PG play important roles in bone homeostasis,[23-25] specifically COX-2, which has a key role in PGE-2 production and is important in osteoblast formation.[25] The effect of PGs on bone metabolism is mediated through the PGE receptor types 2 and 4 (EP2 and EP4). In osteoblast formation, PG could increase the proliferation and differentiation of osteoblasts, while in bone resorption, PG could increase the activity of the osteoclast. The inhibition of cyclooxygenase, particularly COX-2, prevents the elevation of PG levels, molecules related to the induction of inflammation, which can lead to various pain sensations.[26]

Osteogenic differentiation

The good effect of ASA on the enhancement of the osteoblastic potential of derived stem cells has been confirmed by a few studies either *in vitro* or *in vivo*.[27-32] In *in vitro* studies, the experiment begins with cell differentiation of osteogenic derived from various sources such as dental stem cells, BMMSCs, and adipose stem cells using osteogenic media and accompanied by modulations in the expression of multiple osteogenic gene markers.[28,29] The confirmation of osteogenic differentiation can be verified via cellular and molecular levels. The mineralized nodule formations were investigated by ARS staining, following which expression levels of osteogenesis markers were assayed by qPCR or western blot.

Beneficial impact of ASA on enhance osteogenic potential (The aspirin's effect on the differentiation of various types of stem cells)

ASA has been reported to modulate a variety of conditions related to human diseases, such as cardiovascular disease, periodontal health, cancer, and diabetes.[33,34] The impact of ASA on stem cell properties has been reported in a number of studies[27-32,35-37] (Table 1).

Table 1.

The effects of ASA on different stem cell in vitro studies [adapted from Zafarmand et al.[37]]

Differentiation	Cell type	Aspirin		In brief
		Dose (μM)	Duration (days)	
Osteogenesis	PDLSC	250−1000	Up to 21	Facilitation in osteogenesis.
	DPSC	≤560	Up to 21	
	MSC	≤800	Up to 21	
	SHED	≤280	3	
	BM-MSC	280- 800	14	

A study by Wang et al.[27] showed that ASA could promote tenogenesis of tendon stem cells (TSCs) via the SMMAD pathway. The study involved RNA sequencing, and the results showed that GDF6, GDF7, and GDF11 were upregulated in the induction medium with the ASA compared to the induction medium group. GDF7 increased tenogenesis and activated Smad1/5 signaling. This showed that ASA promotes TSC tenogenesis and tendinopathy healing through GDF7/Smad1/5 signaling.

Our previous study[28] showed that ASA at 1,000μM enhances the osteogenic potential of PDLSCs. Using a fold change (FC) of 2.0 as a threshold value, the gene expression analyses indicated that 19 genes were differentially expressed. FGF9, VEGF-A, VEGF-C, and FGF2 were markedly upregulated (FC range, 6 to 15), whereas pleotropin, FGF5, brain-derived neurotrophic factor, and DKK1 were among those markedly downregulated (FC 32). This study showed that ASA modulates the expression of growth factor-associated genes and enhances osteogenic potential in PDLSCs. ASA upregulated the expression of genes that could activate biological functions and canonic pathways related to cell proliferation, human embryonic stem cell pluripotency, tissue regeneration and differentiation. ASA could enhance PDLSCs' function.[29] Thus, ASA enhances PDLSC function and may be useful in regenerative dentistry applications, particularly in the areas of periodontal health and regeneration.

We suggest that ASA acts as a regulator for FGF1 to bind with the FGFRL1 receptor and activates several bone-related marker genes. FGFs are heparin-binding proteins and signal through binding to the tyrosine kinase in the intracellular region of the FGR receptor (FGFR). The FGFRs contain an extracellular ligand-binding domain, a transmembrane region, and an intracellular-divided tyrosine kinase domain. The binding of FGFs to FGFRs enables the autophosphorylation of tyrosine in the intracellular region of FGFR, leading to the activation of intracellular downstream signaling pathways, such as Ras/MAPK, protein kinase B, protein kinase C, phospholipase C, and also the p21 pathways.[38] In addition, Miraoui et al.[39] reported that FGFR2 acts as a novel regulatory

molecule that promotes osteogenic differentiation in murine MSCs. The effect of FGFR2 is mediated by PKCα and ERK1/2 pathways that have critical roles in FGFR2-induced osteogenic differentiation of murine MSCs.

A study by Xie et al.[30] showed that low-dose aspirin (<100 μg/mL), which is widely recommended for the prevention of thrombosis, is very likely to be beneficial for maintaining bone mass and qualities by activation of osteoblastic bone formation and inhibition of osteoclast activities via the cyclooxygenase-independent pathway.

A study by Zhang et al.[35] demonstrated that the tetra-PEG hydrogel-based aspirin sustained release system exerts beneficial effects on PDL SC-mediated bone regeneration. The researchers developed a tetra-PEG hydrogen sealant with rapid gelation speed, strong tissue adhesion, and high mechanical strength to achieve a prognosis of the bone defect. After in situ encapsulation of aspirin, this drug-loaded tetra-PEG hydrogel possessed sustained release, anti-inflammation, and osteoinductive properties. In their study, the viability of PDLSCs was significantly improved in both PEG and PEG-ASA hydrogels at 24 hours, indicating that the hydrogels that were fabricated obtained superior biocompatibility to support PDLSCs. In their study, ASA-loaded PEG hydrogels displayed a slow-release profile that could promote osteogenic differentiation of PDLSC both in vivo and in vitro. This data suggests that ASA can promote the osteogenic differentiation capacity of PDLSCs.

ASA induces growth factors in promoting osteoblastic potential in stem cells

The effects of ASA on various growth factors have been shown to enhance osteoblastic potential of derived stem cells.[28-30,40] ASA could promote the immunomodulatory function of BMMSCs by upregulation of regulatory T-cells and downregulation of Th17-cells via 15-deoxy-delta-12,14-prostaglandin J2/peroxisome proliferator-activated receptor-γ/transforming growth factor-β1 pathway.[40]

FGFs have been isolated from many sources, and although they have a pivotal role in cell proliferation, they also display many functions in the epithelial and mesenchymal cells.[41] Structurally, FGFs contain 22 members, and their molecular weight ranges between 17 to 34kDda.[42] FGFs are divided into two classes: acidic (a-FGF) and basic (b-FGF), and these were originally isolated from the brain and pituitary glands as growth factors for fibroblasts.[42] b-FGFs have shown various biological functions, including development, differentiation, angiogenesis, and wound healing. The expressions of FGFs play major roles in bone development and are found in mesenchymal progenitor and osteoblast cells. In addition, a study has shown that b-FGF, especially FGF2, maintains the proliferation as well as trilineage differentiation capacity in MSCs through the early mitogenic cycles. However, eventually, all the MSCs differentiate into the chondrogenic line.[43] FGF2 was reported to markedly enhance the growth rates and the life spans of MSCs from rabbit, canine, and human bone marrows in monolayer cultures.[44] b-FGF could enhance the levels of cAMP, ALP, OCN, mineralization, and differentiation of osteogenic precursor cells of rat stromal bone marrow cells (rSBMC) isolated from young adult rats. This suggests b-FGF is able to stimulate rSBMC growth and biochemical functions.[45] The exposure of b-FGF to rat BMMSCs enhances *in vitro* osteogenic development in the presence of dexamethasone as the inducer.[46] The treatment of rat BMMSC in combination with b-FGF and BMP2 was also shown to synergistically enhance the osteogenic potential of the stem cells, compared to BMP2 treatment alone.

FGF18 was reported to be expressed in mesenchymal tissues during the differentiation of osteoblasts in calvarial bone development and in the perichondrium of developing bones.[47] It appears to positively affect osteogenesis by regulating cell proliferation and differentiation while at the same time negatively regulating chondrogenesis.[47] The biological activities of FGFs are dependent on the presence of BMP proteins. FGF4 and FGFR signals play important roles during BMP2-induced bone formation, as observed in rats.[48]

FGF signaling controls osteoblast gene expression

FGFs are heparin-binding proteins that function by binding to the following: tyrosine kinase in the intracellular region of FGR receptors (FGFRs), non-transducing heparan sulfate-containing proteoglycans, the cysteine-rich receptor, and binding proteins.[41] FGFRs contain an extracellular ligand-binding domain, a transmembrane region, and an intracellular-divided tyrosine kinase domain.

There are four distinct types of FGFR tyrosine kinase receptors (FGFR1, FGFR2, FGFR3, and FGFR4) with different FGF-binding properties. FGFRs are expressed in many different cell types and regulate proliferation, differentiation, and survival. FGFR1 and FGFR2 are expressed in MSCs prior to deposition on the extracellular bone matrix during bone development, as shown in Figure 1. FGFR1 was recently shown to be an important transducer of FGF2 signals in proliferating osteoprogenitor cells and subsequent differentiation during short-term cultivation.[49]

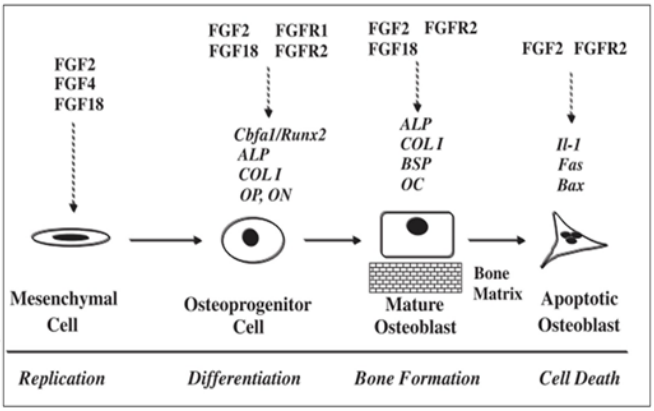

Fig. 1. *Roles of FGF and FGR in osteoblast differentiation and fate. FGF acts through FGFR (dashed arrows) to control genes involved in osteoblast proliferation, differentiation, and apoptosis [**a**dapted from P.J. Marie[3]].*

ALP - alkaline phosphatase; Col I - type I collagen; OP - osteopontin; ON - osteonectin; BSP - bone sialoprotein; OC - osteocalcin

The proliferation of osteoblasts at an early stage allows osteoblastic lineage commitment to take place. FGF signaling

regulates the expression of various genes that are involved in osteoblast differentiation. Its effect on osteoblast marker genes varies, depending on the cell types, either directly or indirectly. The direct effect of FGF signaling is mediated by transcription factors, while the indirect effect is mediated by soluble factors or cell-matrix interactions.[3]

The stimulation of Runx2/Cbfa1 expression by FGF2 provides an important molecular mechanism by which FGF/FGFR signaling directly activates the expression of osteoblast genes that are dependent on Runx2/Cbfa1. FGFs signaling could also have indirect effects on growth factors, matrix degradation, and cell proteins. In such cases, FGF signaling could affect osteoblast differentiation. Another example of the indirect effect of FGF signaling is to regulate osteogenic differentiation through the interaction of the growth factor signaling pathway.

In addition, FGF can also influence other growth factors that influence osteoblast formation, and this could enhance bone formation. For example, FGFs increase VEGF and hepatocyte growth factor (HGF) levels, both of which are mitogenic factors for osteoprogenitor cells.[50] FGFs also regulate the genes involved in matrix degradation (Table 2). FGF signaling regulates the expression of IL6, which is an important mediator of the effects of b-FGF on bone cells.[51,52] FGF2 modulates the bone matrix by regulating the expression of collagenase activity. In fetal rat osteoblast-enriched (Ob) cells, it has been observed that b-FGF can stimulate collagenase-3 gene promoter activity through the AP-1 promoter binding site[53] and stromelysin-3 transcription.

Table 2.

Gene regulation by FGF/FGF signaling in bone [adapted from PJ. Marie[3]]

Transcription factors	Matrix and cell proteins	Growth factors	Matrix degradation	Apoptopic proteins
AP-1 Cbfa1/Runx2 Twist	Col I ON, OP BSP OC N-Cadherin Connexin-43 Noggin	TGFβ IGF1 IGFBP5 VEGF HGF	MMP1 Collagenase-3 TIMPs Stromelysin MMP1	Bax IL-1 Fas

Conclusion

ASA may potentially enhance periodontal regenerative processes by stimulating a selected number of growth factor-associated genes in PDLSCs or/and via its enhancement of osteogenic potential. These observations suggest that ASA could be supportive of regenerative processes and may help to improve periodontal health. However, further in-depth investigations, such as global proteome and transcriptome profiling studies, may provide additional insights on the impact of ASA on the regenerative activities of PDLSCs and how it could affect PDL functions in periodontal health and regeneration.

Competing Interests

The authors declare that they have no competing interests.

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