Role of nitric oxide in orthodontic tooth movement (Review)

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Abstract. Nitric oxide (NO) is an ubiquitous signaling molecule that mediates numerous cellular processes associated with cardiovascular, nervous and immune systems. NO also plays an essential role in bone homeostasis regulation. The present review article summarized the effects of NO on bone metabolism during orthodontic tooth movement in order to provide insight into the regulatory role of NO in orthodontic tooth movement. Orthodontic tooth movement is a process in which the periodontal tissue and alveolar bone are reconstructed due to the effect of orthodontic forces. Accumulating evidence has indicated that NO and its downstream signaling molecule, cyclic guanosine monophosphate (cGMP), mediate the mechanical signals during orthodontic-related bone remodeling, and exert complex effects on osteogenesis and osteoclastogenesis. NO has a regulatory effect on the cellular activities and functional states of osteoclasts, osteocytes and periodontal ligament fibroblasts involved in orthodontic tooth movement. Variations of NO synthase (NOS) expression levels and NO production in periodontal tissues or gingival crevicular fluid (GCF) have been found on the tension and compression sides during tooth movement in both orthodontic animal models and patients. Furthermore, NO precursor and NOS inhibitor administration increased and reduced the tooth movement in animal models, respectively. Further research is required in order to further elucidate the underlying mechanisms and the clinical application prospect of NO in orthodontic tooth movement.

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Abbreviations: NO, nitric oxide; cGMP, cyclic guanosine monophosphate; NOS, nitric oxide synthase; L-arg, L-arginine; nNOS, neuronal NOS; eNOS, endothelial NOS; iNOS, inducible NOS; sGC, soluble guanylyl cyclase; PKG, cGMP-dependent protein kinases; PDE, phosphodiesterase; PDL, periodontal ligament; CGRP, calcitonin gene-related peptide; M-CSF, monocyte/macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; IL, interleukin; TNF, tumor necrosis factor; PGE2, prostaglandin E2; cAMP, cyclic adenosine monophosphate; MMPs, matrix metalloproteinases; Runx2, transcription factor runt-related transcription factor 2; BMP, bone morphogenetic protein; TGF, transforming growth factor; MAPK, mitogen-activated protein kinase; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; FSS, fluid shear stress; ECM, extracellular matrix; Cx, connexin; FAK, focal adhesion kinase; ODQ, 1H-(1,2,4)oxadiazolo-(4,3-a)quinoxalin-1-one; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PFF, pulsed fluid flow; GCF, gingival crevicular fluid; L-NAME, N(G)-nitro-L-arginine methyl ester

Key words: NO, cGMP, orthodontic tooth movement, bone remodeling, osteoblast, osteoclast

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1. Introduction

Nitric oxide (NO) is a water-soluble, gaseous, short-lived free radical molecule that plays multifaceted roles in a broad range of physiological and pathological processes in mammals (1-3). NO is produced by NO synthase (NOS) as a consequence of the process of L-arginine (L-arg) conversion into L-citrulline with the participation of oxygen and nicotinamide adenine dinucleotide phosphate. Three isoforms of NOS have been identified: Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutively expressed calcium-dependent enzymes, characterized by the rapid production of a small amount of NO; inducible NOS (iNOS) is a calcium-independent enzyme that is upregulated at the transcriptional level during

inflammation, causing a relatively slow yet increased-output NO production (2,3). The most common target of NO is soluble guanylate cyclase (sGC), which generates the second messenger cyclic guanosine monophosphate (cGMP) from guanosine-5'-triphosphate within the cell (4,5). cGMP mainly acts on protein kinase G (PKG) and can be degraded by phosphodiesterase (PDE), such as PDE5, 6 and 9 (2,6). The effect of NO on bone mass regulation and bone metabolism has been well investigated and reviewed elsewhere; however, studies on the involvement of NO in orthodontic tooth movement are limited (7-9).

Tooth movement induced by orthodontic force is achieved through bone remodeling, as a result of the sequential transduction of molecular signals and changes in cellular behaviors (10,11). It is of utmost significance to determine the underlying mechanism of orthodontic tooth movement, in order to reduce possible side-effects and shorten the duration of therapy. NO is extensively involved in orthodontic-related biological events, such as aseptic inflammation, mechanical signal transduction and bone remodeling. Furthermore, the regulatory effect of NO on bone remodeling has been demonstrated to be cGMP-related (12,13). In the present review, the regulatory effects of NO on the functional states of related cells and tissues during orthodontic tooth movement, as well as the possible mechanisms involved are discussed, with the aim of providing helpful insight towards the application of effective therapeutic interventions in orthodontics.

2. Orthodontic tooth movement overview

Orthodontic tooth movement relies upon periodontal ligament (PDL) and alveolar bone remodeling. The PDL is a dense connective tissue that plugs the tooth to the adjacent alveolar bone (14,15). It contains collagen fiber bundle, blood vessel, nerves, interstitial fluids and multiple cell types, including fibroblasts, osteoclasts, osteoblasts and macrophages (10,14). The alveolar bone consists of bone cells (osteoclasts, osteoblasts and osteocytes) and the mineralized matrix (14,16). The force applied to the tooth triggers cell-signaling cascades in the PDL and the alveolar bone, leading to tissue remodeling and tooth movement (11,17).

Orthodontic tooth movement can be organized into three phases: i) The initial phase; ii) lag phase; and iii) post-lag phase (18). In the initial phase, tooth movement occurs due to the deformation of PDL and tooth displacement within the alveolar socket 24 to 48 h after the application of force to the teeth. The lag phase follows the initial phase, during which little or no tooth movement is observed due to PDL hyalinization in the compression region. This phase lasts 20-30 days. Following the removal of necrotic tissue by macrophages, tooth movement resumes in the post-lag phase (19,20). This phase usually occurs 40 days after the initial application of force.

Cellular and molecular mechanisms of orthodontic tooth movement

Pressure side: Osteoclasts and bone resorption. The pressure-tension theory describes orthodontic tooth movement as an outcome of bone resorption in the compression region and bone formation in the tension region (21). On the pressure side, the reduction of blood flow and the distortion of nerve endings in PDL may cause hypoxia and the release of vasoactive neurotransmitters, including substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal polypeptide. As a result, vasodilatation and the aggregation of circulating leukocytes, monocytes, macrophages, lymphocytes and mast cells has been observed (22-26). Growth factors, chemokines and other cytokines also contribute to these processes (23,27,28).

Osteoclasts are multinucleated cells, that initially differentiate from multipotential hematopoietic precursors in the monocyte/macrophage lineage, upon macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL) stimulation, which are secreted primarily by cells of the osteoblast lineage (29-35). M-CSF promotes the proliferation, adhesion and migration of osteoclast precursor cells (36-38). RANKL promotes the fusion, differentiation and bone resorptive function of osteoclasts through the activation of RANK on the surface of osteoclast precursors (33,39,40). OPG, a decoy receptor for RANKL, suppresses osteoclastogenesis through the blockage of the RANK/RANKL signaling pathway (41,42).

The aseptic inflammatory response caused by orthodontic forces is indispensable for tooth movement (11,43). Interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and prostaglandin E2 (PGE2) can induce the release of RANKL and MCS-F to stimulate osteoclast precursor differentiation (41,44-47). In addition to the enhancement of osteoclastogenic factor expression, TNF-α also activates osteoclast precursors directly through it binding to TNF receptor (32,48,49). PGE2 enhances the bone-resorbing activity of osteoclasts through the increase of intracellular cyclic adenosine monophosphate (cAMP) levels or the partial mediation of TNF-α (50). Mature osteoclasts occupy small cavities termed Howship's lacunae, in which hydrogen ions and proteolytic enzymes are released, including cathepsin K and matrix metalloproteinases (MMPs), in order to degrade the bone matrix (39,51,52). When the magnitude of the force decreases, osteoclasts become inactive and detach from the bone (53).

Tension side: Osteoblasts and bone formation. Bone deposition induced by osteoblasts presents is the predominant event on the tension side (20,54). Derived from bone marrow mesenchymal stem cells, osteoblasts secrete an organic matrix known as the osteoid, which is then incorporated further into the mature bone (55). During bone formation, some osteoblasts transform into bone lining cells on the bone surface, or osteocytes embedded in the bone matrix. Osteocytes are connected and communicate through cytoplasmic processes in tiny canals, called canaliculi (56,57).

Transcription factor runt-related transcription factor 2 (Runx2), also known as ore-binding factor subunit alpha-1 (Cbfa1) and the Wnt/β-catenin pathway provide the initial and essential stimulus for osteoblast differentiation (34,58). Bone morphogenetic protein (BMP), as a member of the transforming growth factor β (TGF-β) superfamily, induces the differentiation of osteoprogenitor cells and promotes osteoblast function through the stimulation of Runx2 expression via the small mother against decapentaplegic or p38

mitogen-activated protein kinase (MAPK) pathways (59-62). In addition, TGF-β also suppresses bone resorption activity through the upregulation of the tissue inhibitor of metalloproteinases expression (43,63). IL-10 induces an overall reduction in RANK signaling, through the facilitation of OPG expression and the reduction of RANKL production (43,64,65).

Regional hypoxia caused by orthodontic force induces hypoxia-inducible factor (HIF)-1 expression and upregulates the transcription of vascular endothelial growth factor (VEGF) in PDL fibroblasts and osteoblasts. VEGF is associated with osteogenic differentiation and matrix mineralization under the regulation of BMP, corroborating the concept that angiogenesis and osteogenesis are combined (66,67). Furthermore, HIF-1 and VEGF also stimulate osteoclast differentiation via the upregulation of RANKL, contributing to the combination of bone resorption and bone formation (68-70).

Some molecules that regulate the response of PDL fibroblasts to the orthodontic forces have been identified in previous studies, such as CC chemokine receptor 5 (CCR5) and CCR5 ligands axis (71), relaxin (Rln) and Rln family peptides (Rxfps) axis (72), and secretory leucocyte peptidase inhibitor (73). The expression levels of these molecules were upregulated in the PDL, due to compression and tension force; however, their downstream effects were different. Another consequence was the upregulation of the osteoclastogenesis-relating factors, including RANKL, MCSF and MMPs, on the compression side, and osteoclast activity inhibiting factors, including Runx2, IL-6, and IL-12, that may induce osteoblast differentiation on the tension side.

Mechanotransduction: Osteocytes and fibroblasts. Osteocytes are critical for the transduction of mechanical stimuli into biochemical signals (74-76). When a force is exerted on the tooth, the squeeze of the interstitial fluid causes fluid shear stress (FSS) in the extracellular matrix (ECM) (77). The fluid flow hypothesis describes the response of osteocytes to FSS as an essential mechanism during orthodontic treatment. FSS stimulates an increase in the intracellular calcium concentration and the release of intercellular molecules in osteocytes through the activation of integrin, a transmembrane protein that connects ECM macromolecules to the internal cytoskeleton (78-80). The FSS-related up-regulation of NO, PGE2, TFG-β, and insulin-like growth factor alters the osteocyte metabolic state and osteoblast/osteoclast functions (81,82). Gap junctions formed by connexin (Cx) also participate in the osteocyte-osteoblast communication (83,84). For example, Cx is involved in the release of PGE2, which enhances Runx2 DNA binding activity through the simultaneous activation of the cAMP/cAMP-dependent protein kinase and MAPK pathways and the subsequent stimulation of RANKL expression in osteoblasts (85-88).

The inhibitory effect of osteocytes on osteoblastic activity can be induced by the secretion of sclerostin, which antagonizes BMP effect and blocks canonical Wnt signaling (89-91). Osteocytes regulate osteoclastic differentiation via the alternation of major osteoclast regulators, namely RANKL and M-CSF (92-94). Moreover, osteocyte apoptosis induction is an important event in the recruitment and differentiation of osteoclasts (95-97). These findings confirm that osteocytes play a key role in the response to biomechanical stimuli and controlling bone remodeling by coordinating the activity of osteoblasts and osteoclasts.

Fibroblasts are involved in mechanosensation and mechanotransduction in connective tissues. The application of mechanical stretching activates integrin and causes conformational changes in focal adhesion kinase (FAK), inducing a signaling cascade that modulates cytoskeletal dynamics and gene transcription in fibroblasts (98,99).

3. Effects of NO on orthodontic tooth movement

Expression of NO in bone tissue. Three NOS isoforms in total are expressed in osteoblasts, osteoclasts and osteocytes (100-102). iNOS and eNOS are expressed in human PDL stem cells (103,104). Previous studies revealed the presence of sGC and cGMP in mouse bone marrow macrophages (105), osteoclasts (105-107), and osteocytes (108). Davidovitch *et al* (109,110) performed immunohistochemistry (IHC) on alveolar bone sections obtained from cats and revealed that cGMP expression was increased in the PDL fibroblast cells stained intensely for; however, most cGMP expression was not detected through IHC staining in osteoblasts. However, cGMP expression increased due to the subjection of the alveolar bone to mechanical force (111,112). The application of electric currents to the bone, also led to the upregulation of cGMP in osteoblast and PDL fibroblast cells, accompanied by bone deposition near the cathode (113-115). Since a piezoelectric current can be generated by mechanical stress, the above findings suggest that NO/cGMP is an important signaling pathway, which mediates bone cell response to mechanical force (116).

Role of NO in cells associated with orthodontic tooth movement. Mounting evidence indicates that NO regulates multiple cellular behaviors related to orthodontic movement (Fig. 1 and Table I).

Osteoclasts. A number of studies have demonstrated that NO exerts biphasic effects on osteoclast formation and function. In several cases, NO promotes osteoclastogenesis and bone resorption. NO mediates pre-osteoclasts fusion through the upregulation of actin cytoskeleton remodeling (117). Histopathological studies have demonstrated that osteoclasts, Howship's lacunae and new capillaries were increased in rats that received an injection of the NO precursor L-arg during tooth movement (118-120).

iNOS is an important regulator of osteoclast differentiation under bacterial infection-induced inflammatory conditions (121-123). iNOS was previously found to mediate alveolar bone loss and periapical infectious bone resorption following the oral administration of *Porphyromonas gingivalis* (124) or lipopolysaccharide (122). In another study, histochemical analysis revealed that the osteoclast number in iNOS(-/-) mice in comparison to wild-type mice was considerably decreased (123). Tooth eruptions are similar to tooth movement in terms of monocyte recruitment and osteoclast differentiation. Evidence indicates that increased levels of iNOS are associated with a greater number of osteoclasts in mice with accelerated tooth eruption, indicating that iNOS may be a bone resorption modulator candidate (125).

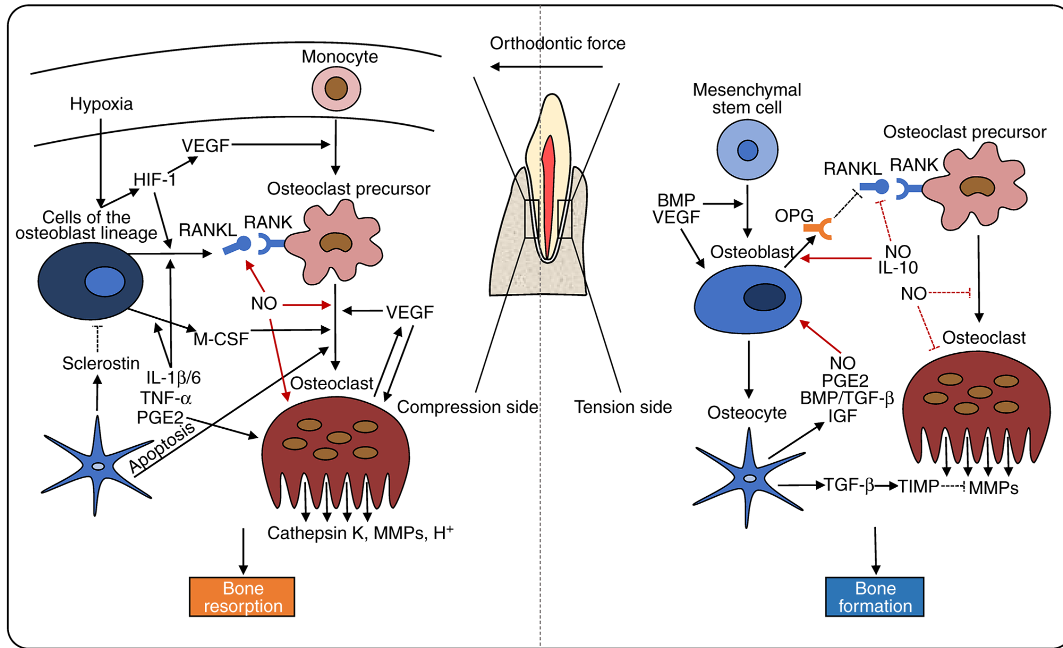

Figure 1. A schematic representation of bone remodeling and NO regulation during orthodontic tooth movement at the compression and tension sides. After orthodontic force is applied to the teeth, bone remodeling in the compression region mainly manifests as osteoclastogenesis and bone resorption, while that in the tension region presents as osteogenesis and bone formation. The regulation of related factors on bone remodeling is indicated by black arrows. NO regulation of osteoclasts and osteoblasts differentiation is indicated by red arrows. HIF-1, hypoxia-inducible factor-1; VEGF, vascular endothelial growth factor; RANKL, receptor activator of nuclear factor-κB ligand; RANK, receptor activator of nuclear-κB; M-CSF, macrophage-colony stimulating factor; IL-1β/6, interleukin-1β/6; TNF-α, tumor necrosis factor-α; PGE2, prostaglandin E2; MMPs, matrix metalloproteinases; BMP, bone morphogenetic protein; OPG, osteoprotegerin; TGF-β, transforming growth factor-β; IGF, insulin-like growth factor; TIMP, tissue inhibitor of metalloproteinases.

As previously demonstrated, M1-like macrophage polarization and an enhanced M1/M2 macrophage ratio increase the number of osteoclasts in rats or mice, accompanied by an increase in M1 macrophage marker expression (TNF-α and iNOS) on the compression side, during tooth movement (126,127). TNF-α stimulates the survival of differentiated osteoclasts through the induction of iNOS-dependent NO generation (128). In the rheumatism inflammatory environment, the TNF-α promoting effect on alveolar bone resorption is partly mediated through the activation of iNOS and the resulting production of NO (129).

It has been observed that the promoting effect of iNOS on osteoclasts is mediated through the NO/cGMP pathway. Kaneko *et al* (105) revealed that 8-nitro-cGMP, a NO-dependent derivative of cGMP in mammals, increased RANKL mRNA expression, and enhanced osteoclast differentiation. The reduction in cGMP levels due to the inhibition of NOS caused RANKL-induced osteoclast differentiation suppression.

By contrast, evidence has revealed an inhibitory effect of NO on osteoclasts at low concentrations. NO has been reported to increase osteoclast and osteoclast precursor cell apoptosis (101,130-132). A novel NO donor, nitrosyl-cobinamide (NO-Cbi), has been found to reduce the RANKL/OPG gene expression ratio or directly inhibit osteoclast differentiation *in vitro* and *in vivo* (133). Nicorandil, an agent that can increase NO production in osteoclasts, was previously shown to suppress osteoclast differentiation via activating sGC (134). NO causes osteoclast detachment and downregulates osteoclast bone-resorbing activity via the NO/cGMP/PKG pathway *in vitro* (101,107,135-137). Of note, the selective inhibition of iNOS was previously found to markedly promote bone resorption *in vivo*. In an iNOS(-/-) mouse model of apical periodontitis, enhanced osteoclast differentiation and increased bone resorption were observed in comparison with the control group, accompanied by increased IL-1β, TNF-α, RANK, RANKL and monocyte chemoattractant protein-1 (MCP-1) levels (138,139). These results suggest that NO deficiency is associated with an imbalance in the host inflammatory response, resulting in severe bone loss.

Moreover, iNOS exerts an inhibitory effect on osteoclast differentiation through other pathways. Zheng *et al* (140) demonstrated that iNOS was a RANKL-induced autocrine negative feedback inhibitor of RANKL-mediated osteoclastogenesis. RANKL triggered iNOS expression and NO release, and subsequently inhibited RANKL-induced osteoclast formation in a cGMP-independent manner.

The inconsistent effects of NO on osteoclastogenesis may be attributed to the differences in NO synthesis quantity, cell types and development states. NO action is also affected by the cytokines in the microenvironment. Multiple factors influence the downstream signaling of NO, and further studies are required to elucidate the specific mechanism of NO regulation.

Osteoblasts. NO is also involved in the bidirectional regulation of osteoblasts. Decreased NO concentrations promote osteoblast proliferation, differentiation and survival (133,141-143). Mineralized nodule formations and mRNA expression levels of osteoblastic genes, such alkaline phosphatase, osteocalcin and collagen-1 genes, have been shown to be enhanced by NO donors and 8-Br-cGMP, an analog of cGMP (141-143). This effect was blocked by 1H-(1,2,4)oxadiazolo-(4,3-a)quinoxalin-1-one (ODQ), a competitive blocker that prevents sGC

Table I. Role of NO signaling in the regulation of cells related to orthodontic movement.

Cell type	Agent (concentration)	Regulation	Downstream pathways	(Refs.)
Murine osteoclasts	8-Nitro-cGMP (30 μM)	Promoted osteoclast formation	Enhances the mRNA expression of RANK via PKG	(105)
RAW264.7 murine osteoclasts	NOC-12 (15 μM), NOC-18 (5 μM) NOC-12 (>25 μM), NOC-18 (>10 μM)	Promoted osteoclast formation Decreased osteoclast survival	Regulated actin cytoskeleton remodeling and pre-osteoclast fusion	(117)
RAW264.7 murine osteoclasts	AG (2-500 μM)	Promoted osteoclast formation	RANKL/IFN-β-induced iNOS/NO as a negative feedback signal during osteoclastogenesis	(140)
UMR-106 and MC3T3-E1	SNAP (0-1000 μM)	Promoted osteoclastic activity	Augmented the TNF-α-stimulated MMP-1 mRNA	(122)
Murine osteoclasts	NOC-18 (10-500 nM) NOC-18 (1-50 μM)	Increased osteoclast survival	Mediated the TNF-α-induced osteoclast survival by reducing the activity of caspase 3	(128)
Rat osteoclasts	YC-1 (100 nM)	Decreased osteoclast survival Inhibited osteoclast activity	Activated caspase-3/caspase-8 activity and inhibited Src activity	(130)
Murine osteoclasts	SNAP (300 μM)	Decreased osteoclast survival	Mediated apoptosis of osteoclast progenitors induced by TNF-α and IFN-γ	(131)
Murine osteoclasts	L-NMMA (0.1-10 mM)	Increased osteoclast survival	Mediated cell apoptosis of osteoclast progenitors induced by IL-12 and IL-18	(132)
Murine osteoclasts	NO-Cbi (3-30 μM)	Inhibited osteoclast formation	Reduced the RANKL/OPG gene expression ratio	(133)
Murine osteoclasts	Nicorandil (1-100 μM)	Inhibited osteoclast formation	Via cGMP	(134)
Human osteoclasts	SNAP (20 mM) SNP (100 μM)	Inhibited osteoclast activity	Downmodulated acid secretion and inhibited integrin attachments via cGMP/PKG I/VASP/IP3R1/IRAG	(135-137)
FLG 29.1 human preosteoclast cell line	SIN-1 (50-200 μM)	Decreased cell proliferation	-	(101)
Avian osteoclasts	SNP (unknown)	Inhibited osteoclast activity	Reduced osteoclast membrane HCI transport activity via PKG	(107)
Murine osteoblasts	NO-Cbi (3-30 μM)	Promoted osteoblast proliferation and differentiation	Stimulated ERK/Akt and Wnt/β-catenin signaling via cGMP/PKG	(133)
Rat osteoblasts	NOC-18 (10 μM)	Promoted osteoblast differentiation	Via cGMP	(141)
Murine osteoblasts	SNP (0.01 μM -1 mM) DEA-NO (0.1-100 nM)	Promoted osteoblast differentiation	Via cGMP	(142)
Rat osteoblasts	8-Br-cGMP (10-100 μM)	Promoted osteoblast differentiation	-	(143)
Murine osteoblasts	Sildenafil and vardenafil (10 nM-1 mM)	Promoted osteoblast differentiation	Increased the expression of VEGF and VEGFR2	(144)
Rat osteoblasts	NOC-18 (10-50 μM), SNP (100 μM)	Promoted osteoblast survival and differentiation, decreased osteoblast survival	Via cGMP	(145)
MC3T3-E1 osteoblasts	DEA-NO (100 μM)	Promoted osteoblast differentiation	Regulated MMP-13 expression via cGMP/PKG/Runx2	(146)
MC3T3-E1 osteoblasts	SNP (1.5-3 mM)	Decreased osteoblast survival	Increased expression levels of p62, ATG7, Beclin-1 and LC3-II via AMPK	(155)

Table I. Continued.

Cell type	Agent (concentration)	Regulation	Downstream pathways	(Refs.)
MLO-Y4 murine osteocyte-like cells	DETA-NONOate (3 μM)	Increased osteocyte survival	Mediated the effects of estradiol by activating Akt/ERK and phosphorylating BAD via PKG Iα and PKG II	(157,158)
Human PDL cells	SNP (0.5-1.0 mM)	Decreased cell proliferation Promoted cell differentiation	Via HO-1/ERK/NF-κB	(159)
Human PDL stem cells	SNP (75 μM)	No influence on proliferation and survival, promoted osteogenic and reduced adipogenic differentiation	Via JNK MAPK	(103)
Human PDL fibroblasts	SNP (1-4 mM)	Decreased cell survival	Increased Bax and cytochrome *c*, and reduced caspase-3 via JNK, ERK and p38 MAPK	(162)

DETA-NONOate/DEA-NO/NOC-18, 2,2'-(hydroxynitrosoydrazino)bis-ethanamine; AG, aminoguanidine; SNAP, S-nitroso-N-acetyl-penicillamine; IFN-β, interferon β; YC-1, 3-(50-hydroxymethyl-20-furyl)-1-benzyl-indazole; L-NMMA, NG-methyl-L-arginine; NO-Cbi, nitrosyl-cobinamide; SNP, sodium nitroprusside; VASP, vasodilator-stimulated phosphoprotein; IP3RI, inositol 1,4,5-trisphosphate receptor I; IRAG, IP3RI-associated protein; SIN-1, 3-morpholinosydnonimine; ATG7, autophagy related 7; LC3-II, light chain 3-II; DEA-NO, diethylamine NONOate; HO-1, heme oxygenase-1; Bax, BCL2-associated X protein; JNK, c-Jun N-terminal kinase.

activation and lowers cGMP/PKG activity. It has been recently stated that PDE5 inhibitors, which can significantly increase intracellular cGMP levels, induce osteoblast differentiation and enhance bone regeneration in osteopenic mice via the cGMP/VEGF pathway (144). These findings further support the involvement of NO/cGMP/PKG pathway in the regulation of osteoblast activity (133,143,145).

Increased iNOS expression and NO levels have been observed during osteoblast differentiation *in vitro*. iNOS has been reported to mediate the regulation of Runx2 translocation and downstream events (146). In eNOS knockout mice, osteoblast growth has been shown to be inhibited (147). Evidence suggests that eNOS activation promotes cell survival and enhances osteoblastic gene expression in osteoblasts via pathway cascades involving Src/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) and Wnt/β-catenin (148,149).

NO mediates the action of several local and systemic factors, including mechanical stimulation, hormones and other signaling molecules in osteoblasts (13,150). It has also been revealed that 1,25-dihydroxyvitamin D(3) regulates bone mass via the upregulation of iNOS expression and NO production (151). CGRP has been found to promote mandibular bone fracture healing *in vivo* and stimulate the eNOS activity through the increase of intracellular calcium concentrations in osteoblasts *in vitro* (152,153). Furthermore, it has been observed that 17 β-estradiol, a major endogenous estrogen, may promote eNOS expression and osteoblast differentiation through Akt phosphorylation in a dose-dependent manner (154). It has been previously demonstrated that the bone-protective effects of estrogen rely upon the NO/cGMP pathway (147,150). High concentrations of NO negatively impact osteoblast proliferation and survival (145). NO simultaneously induces cell death and autophagy in osteoblasts (155).

Osteocytes and PDL fibroblasts. The effect of NO on osteocytes is similar to that of osteoblasts. Parathyroid hormone and 17β-estradiol levels increase the expression of cGMP expression in osteocytes (108). Cinaciguat, an activator of sGC that has been declared as a potential drug target for osteoporosis, was previously found to reverse osteocyte apoptosis and enhance bone formation in mice subjected to ovariectomy (156). NO/cGMP/PKG signaling mediated 17β-estradiol anti-apoptotic effect on osteocytes through either the activation of the pro-survival kinases, ERK and Akt, mediated by type II PKG, or direct phosphorylation of protein related to cell death by type I (PKG) (157,158). However, inflammation-induced iNOS activation and elevated concentrations of NO can lead to osteocyte apoptosis (132).

NO/cGMP/PKG signaling has been shown to regulate human PDL fibroblast proliferation and differentiation, with the involvement of MAPK and nuclear factor κ-light-chain-enhancer of activated B cells pathways (103,159-161). However, the effect of NO on cell proliferation in PDL has not yet been fully clarified. A previous study revealed that NO did not influence PDL stem cell proliferation (103). In other studies, it has been revealed that exogenous NO inhibits proliferation and induces apoptosis of PDL fibroblasts (159,162). This discrepancy could be attributed to differences in the cellular differentiation levels and varying applied agent concentrations.

The PDL and the alveolar bone are developed from the dental follicle during tooth development. The literature was reviewed and it was observed that studies of NO regulation impact upon the dental follicle during tooth development has not been reported yet, to the best of our knowledge. It was surmised that the exploration of the underlying mechanism of NO on the development of the PDL and alveolar bone may provide novel insights into the role of NO in the tissue remodeling observed during orthodontic tooth movement.

NO signaling in bone mechanotransduction NO is a factor that mediates early cellular response to applied mechanical forces in the PDL and bone (Fig. 2) (13,163,164). NO synthesis in osteoclasts (165), osteoblast (116,151,163,165-167), osteocytes (164,168), PDL fibroblasts (169), fibroblasts (170), and dental pulp cells (171,172) increased following the application of mechanical loading, pulsed fluid flow (PFF), electrical stimulation, or pulsed electromagnetic field stimulation.

The mechanical loading-induced activation of the Wnt/β-catenin pathway is an important signaling event in osteoblasts, osteocytes, and PDL fibroblasts, and is mediated by a NO-dependent mechanism involving the FAK, Src/ERK and PI3K/Akt signaling pathways (169,173,174). PFF increases NO synthesis in osteoblasts, resulting in PKG II-dependent activation of Src and PI3K-dependent phosphorylation of Akt. The nuclear translocation of β-catenin is induced and the gene expression of c-fos is upregulated, initiating a proliferative response in mechanically stimulated osteoblasts (175-177). When the osteoblast and osteocyte cytoskeleton system of disrupted, PFF-induced NO production is affected (178).

PFF induces the release of multiple soluble factors that promote osteogenesis and inhibits bone resorption. This process is partially dependent on the generation of NO (74,179-181). PFF-induced NO inhibited osteocyte apoptosis through the downregulation of B-cell lymphoma-2 (Bcl-2) and caspase-3 (182). NO also modulates mechanically induced VEGF expression, contributing to angiogenesis during bone remodeling (183,184).

The main NOS isoform that produces NO in osteoblasts and osteocytes under the mechanical force action has not yet been elucidated. The activation of eNOS is associated with the phosphorylation or dephosphorylation at several functional sites on eNOS, which may be induced by FSS, estrogens, VEGF and insulin (185-187). Several studies have revealed that FSS-induced NO production is attributed to the calcium-dependent eNOS activation in bone cells (13,185,188,189). It has been revealed that the occlusal force led to iNOS and eNOS increased expression in hypofunctional and normal PDL fibroblasts (100,104).

Additionally, it has been suggested that eNOS may be not indispensable for mechanically-induced NO synthesis in cultured osteoblasts or eNOS (-/-) mice (190,191). It has also been mentioned that ultrasound-induced bone formation may be mediated through nNOS and iNOS upregulation in osteoblasts (82,163,192,193). Furthermore, osteopontin has been shown to suppress the osteoblast response to ultrasound by inhibiting the expression of nNOS and iNOS through FAK downregulation (194). This inconsistency may be explained in view of the possibility of an alternative way of NO production induction by other NOS isoforms and through a non-enzymatic NO production manner (including reduction of nitrite and denitrosylation of some proteins), in case a specific NOS isoform is absent (195-197). The aforementioned ultrasound results can only prove the role of nNOS or iNOS in ultrasound-induced promotion on osteoblasts; however, those results do not contradict the involvement of eNOS.

These findings suggest that NO plays a complex role in mechanotransduction under stress in the periodontal tissue, and further research on this topic is required.

Effects of NO in orthodontic tooth movement. Many studies have focused on the differential expression of NOS isoforms between areas of compression and tension during orthodontic tooth movement. Experiments in rats revealed that the changes in NOS activity in the PDL could be detected as soon as 1 h after teeth were subjected to orthodontic force (198). The increased expression of iNOS on the pressure side and eNOS on the tension side was observed 24 h after initiating mechanical loading, while increased nNOS expression mainly occurred after 3 h (199). An increase of iNOS-positive osteocytes in the compression area was detected 6 h after force application, while eNOS-positive osteocytes in the tension area increased after 24 h (200). As indicated above, it is generally accepted that iNOS dominates bone resorption at the compression site while eNOS mediates the osteogenic effect in the tension area (200,201).

The availability of studies related to the changes in NO levels in human periodontal tissues before and after orthodontic treatment is limited. Analysis of gingival tissue collected from orthodontic patients revealed that eNOS and iNOS levels increased dramatically 2 weeks after the appliance placement (202). A variety of biomarkers in gingival crevicular fluid (GCF) are often analyzed, in order to facilitate the improvement of clinical treatment. In various studies, many of which recent, it has been mentioned that NO expression levels in GCF is related to orthodontic treatment (203-206). Ford *et al* (203) revealed that NO concentration in GCF on the compression side of the central incisor increased significantly 1 h after the application of fixed orthodontic appliances. In patients who received rapid maxillary expansion therapy, the NO levels in GCF were elevated on day 1 and 10 and were still elevated after 3 months of retention (204,205). However, no significant difference was detected in NO levels in GCF on the tension side, during the above treatment. These results further support the different regulatory effects of NO on the tension and pressure side, which are related to the presence of different NOS isoforms on different sides.

The role of NO in orthodontic treatment has also been confirmed in animal experiments. Tooth movement was markedly promoted in rats that received L-arg injection, whereas a significant reduction of tooth movement was observed in the L-NAME (eNOS inhibitor) group. Histological results also revealed a greater number of osteoclasts in the group with greater tooth movement (119,120,207). Notably, decreased force-induced root resorption was noted in this group in comparison with the control group, although the number of osteoclasts increased in the L-arg injection group (119).

Influences of NO and oral microbiota on the orthodontic tooth movement are also notable. In addition to being synthesized by the body, NO can be produced by oral

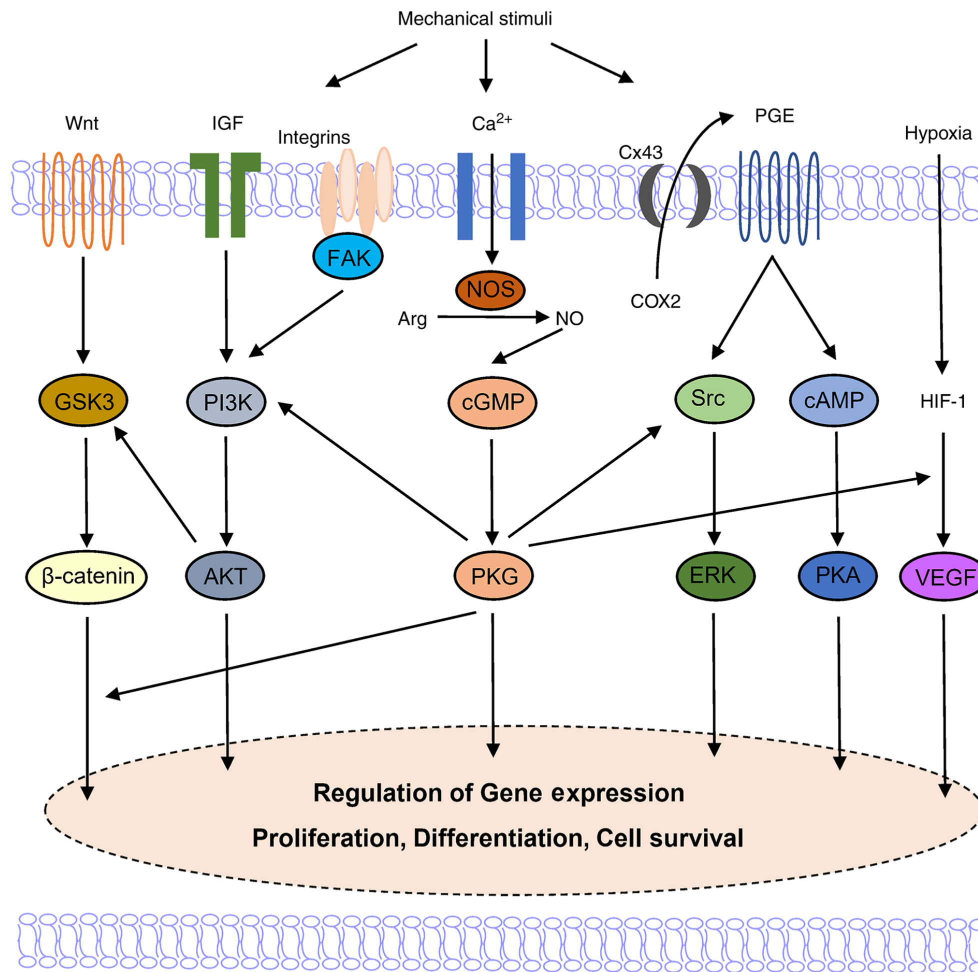

Figure 2. A schematic representation of signaling pathways activated by mechanical stimuli and mechanically-induced NO regulation in osteoblastic cells. Mechanical loads induce signal transduction through the activation of several signaling pathways, resulting in the increased expression of pro-osteogenic factors, thus providing an environment which contributes to osteoblast proliferation and differentiation. NO/cGMP/PKG pathway is widely involved in the regulation of the above signaling pathways, indicating its important role in the mechanical transduction process. GSK3, glycogen synthase kinase 3; IGF, insulin-like growth factor; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; NOS, nitric oxide synthase; Arg, arginine; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; Cx43, connexin 43; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; ERK, extracellular signal-regulated kinase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; HIF-1, hypoxia-inducible factor 1; VEGF, vascular endothelial growth factor.

bacteria under hypoxic conditions through the transformation of saliva nitrate into nitrite (208-210). It has been observed that NO production is upregulated during the deposition of dental plaque (211). In diseases related to plaque accumulation, including periodontitis, an increase in NO levels in both blood and saliva was reported (212-214). Additionally, apart from the oral bacteria-originating NO production, this has also been ascribed to the inflammatory response of the body. It has been previously demonstrated that an enhanced osteoclast formation and accelerated orthodontic tooth movement may be observed in patients with periodontitis (215). It is reasonable to speculate that NO may be involved in this process, but more direct evidence is necessary in order to confirm this (203-207).

4. Conclusions and future perspectives

NO is widely involved in the biomechanical response of the periodontium to orthodontic forces. NO exerts dose-dependent and biphasic effects on the functional status and cell fate determination of osteoblasts, osteoclasts, osteocytes, and PDL fibroblasts, and has been shown to promote the proliferation, differentiation, or inhibition of survival and function of cells. As an inflammatory factor and a key second messenger in mechanical transduction, NO is differentially expressed on the tension and compression side during tooth movement, suggesting its complex involvement in bone remodeling. The facilitation of NO precursor and the inhibition of NOS inhibitor in orthodontic tooth movement have also been confirmed in animal experiments. Additional studies are required, in order to evaluate the role and impact of NO on tooth movement in clinical practice. As NO exerts complex effects on both osteoblastic and osteoclastic activities, the spatiotemporal generation of NO may determine its specific biological effect on bone remodeling. The precise and controlled delivery of NO to periodontal tissue via NO-releasing polymeric nanomaterials may be a promising approach for the acceleration of orthodontic tooth movement.

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Authors' contributions

TY, YX and FH conceived the review. TY performed literature search and manuscript writing. YX contributed to the manuscript writing and the preparation of figures and tables. HH, WF and FH revised the manuscript. TY and FH confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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