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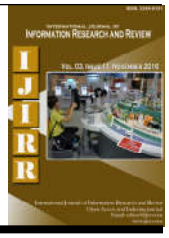

Research Article

BIOMARKERS: ISSUES OF SMALL TISSUES

[1,*]Dr. Harsha P Mahajan, [2]Dr. Rajan Mahindra, [3]Dr. Shubhendra S. Khandewale and [4]Dr. Trupti Agrawal

[1]Dental Surgeon, Dept of Orthodontics, Government Dental College, Aurangabad
[2]Professor and Head of the Department, Dept of Orthodontics, Government Dental College and Hospital, Aurangabad
[3]Lecturer, Dept. of Pedodontics and Preventive Dentistry, Chatrapati Shahu Maharaj Shikshan Sanstha's Dental College, Aurangabad
[4]Assistant Professor, Dept of Prosthodontics, Government Dental College, Aurangabad

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ABSTRACT

Orthodontic tooth movement (OTM) is a complex biological phenomenon involving bone and periodontal tissue. Numerous substances known as biomarkers are released from the dental tissues during this complex procedure leading to a series of biochemical reactions and ultimately orthodontic tooth movement. A thorough knowledge of these biomarkers is essential in order to understand the biological process of tooth movement. It is of advantage to the orthodontist to know the biological events that unfold during tooth movement because it varies from person to person .This review article presents a detail account of physiologic response to orthodontic force and the chemical mediators involved in this process.

INTRODUCTION

Orthodontic tooth movement is composed of three phases: initial tipping, lag phase and progressive tooth movement. Initial tipping occurs when a force (tipping) is applied to a crown of a tooth. The periodontal ligament (PDL) is compressed near the alveolar marginal on the side toward which the tooth is moved. On the opposite side, the PDL is widened or is under tension. The amount of tipping is dependent on the PDL width, root length, anatomical configuration, force magnitude and periodontal health. Orthodontic tooth movement is based on force induced paradental tissue remodeling (Figure 1). Bone resorption by compression-associated osteoclasts and bone deposition by tension-associated osteoblasts are the well-described typical histological characteristics of this process. In the course of tooth movement, root resorption represents a negative side effect. This article provides a detail insight of the current biomedical literature on processes in OTM

Corresponding author: Dr. Harsha P Mahajan
Dental Surgeon, Dept of Orthodontics, Government Dental College, Aurangabad.

The sequence of events after the application of mechanical forces with the help of orthodontic appliances is outlined as (Physiologic response to orthondontic force, 2007):

- Movement of PDL fluids from areas of compression into areas of tension
- A gradual development of strain in cells and Extracellular matrix (ECM) in the paradental tissues involved
- Release of phospholipase A2 and cleavage of phospholipids leading to release of PGE2 and leukotrienes
- ECM remodelling and signal transduction through integrin trans-membrane channels
- Cytoplasmic alterations and release of 2 nd messengers of tooth movement - cAMP and cGMP, ionositol phosphates, calcium and tyrosine kinases
- Release of kinases such as protein kinase A, kinase C and Mitigen activated protein MAP kinases
- Direct transduction of mechanical forces to the nucleus of strained cells through the cytoskeleton, leading to activation of specific genes

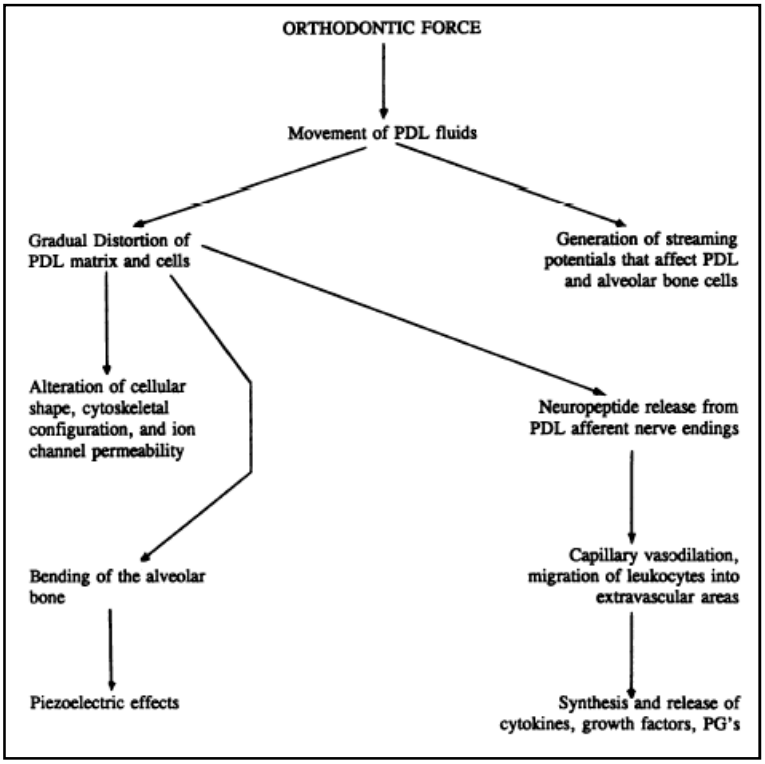

Figure 1. Initial effects of Orthodontic force on paradental tissue. Taken from D. Cardaropoli and L. Gaveglio. The influence of orthodontic movement on periodontal tissues level *Seminars in Orthodontics* 2007; 13: 234–245

Table 1. Physiologic response to orthondontic force. Taken from: Proffit W, Fields H, Sarver D. Contemporary Orthodontics. 4th Edition. St. Louis. Mosbey Elsevier; 2007

Physiologic response to Orthodontic force (Proffit et al., 2007)

Low pressure	High Pressure	Change of event
< 1 sec		P.D.L fluid incompressible, alveolar bone bends piezoelectric signal generates.
1 –2 sec		P.D.L fluid expressed, tooth moves & in P.D.L space.
3 –5 sec		Blood vessels within P.D.L partially compressed on pressure side, dilated on tension side. P.D.L fibres and cells mechanically distorted.
In minutes		Blood flow altered, O2 tension begins to Change, prostaglandins & cytokinins released.
In hours		Metabolic changes occuring, chemical messengers affect cellular activity, enzyme levels change.
In 4 hours		Increased CAMP levels, cellular differentiation begins within P.D.L
In 2 days		Tooth movement beginning as osteoclasts / osteoblasts starts remodeling bony socket.
	3 –5 sec	Blood vessels within P.D.L occlused on pressure side.
	Minutes	Blood flow cut off to compressed P.D.L area.
	Hours	Cell death in compressed P.D.L area.
	3 –5 days	Cell differentiation in adjacent narrow spaces, undermining resorption begins.
	7 –14 days	Undermining resorption removes lamina dura adjacent to compressed P.D.L, tooth movement occurs.

Table 2. Bone Formation Markers. Taken from, Ivana C, Dubravka C: Biochemical markers of bone remodeling – review. Biochemia Medica 2009; 19(1):17-35

Marker	Tissue origin	Analytical sample	Analytical method
Total Alkaline Phosphatase (ALP); specific for bone formation only in patients with no liver or bile duct disease	bone, liver	serum	colorimetry
Bone alkaline phospatase (B-ALP); specific osteoblast product; some procedures show cross reactivity with ALP liver isoenzyme	bone	serum	colorimetry, electrophoresis, precipitation, IRMA, EIA
Osteocalcin (OC, BGP); specific osteoblast product; there are several reactive forms in blood; some can NASTATI during bone resorption	bone, thrombocytes	serum	RIA, ELISA, IRMA, ECLIA
C-terminal propeptide of type I procollagen (PICP); specific proliferating osteoblast and fibroblast product	bone, skin, soft tissues	serum	RIA, ELISA
N-terminal propeptide of type I procollagen (PINP); specific proliferating osteoblast and fibroblast product; partially incorporated into skeletal matrix	bone, skin	serum	RIA, ELISA

IRMA – immunoradiometric assay; EIA – enzyme immunoassay; RIA – radio immuno assay; ELISA – enzyme-linked immunosorbent assay; ECLIA – electrochemiluminiscence immunoassay

Table 3. Bone Resorption Markers. Taken from, Ivana C, Dubravka C: Biochemical markers of bone remodeling – review. Biochemia Medica 2009; 19(1):17-35

Marker	Tissue origin	Analytical sample	Analytical method
Hydroxyproline, total and dialyzable (OH-Pro, OHP); specific for all fibrilar collagens and a part of collagen proteins, including Ciq and elastin; present in newly synthesized and mature collagen	bone, skin, cartilage, soft tissues	urine	colorimetry, HPLC
Pyridinoline (PYD, Pyr); high concentrations in cartilage and bone collagen: not present in skin; present only in mature collagen	bone, tendon, cartilage	urine	HPLC, ELISA
Deoxypyrindoline (DPD, d-Pyr); high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen	bone, dentine	urine	HPLC, ELISA
Cross-linked C-terminal telopeptide of type I collagen (ICTP); high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen	bone, skin	serum	RIA
Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX); in type I collagen; probably high proportion from bone collagen	all tissue containing type I collagen	urine, serum	ELISA, RIA, ECLIA
Cross-linked N-terminal telopeptide of type I collagen (fragments NTX); in type I collagen; big proportion from bone	all tissue containing type I collagen	urine (alpha/beta), serum (only beta)	ELISA, RIA, ICMA
Hydroxylysine-glycosides (Hyl-Glyc); collagens and collagen proteins; glucogalactosyl- hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil-OHLys is highly represented in bone collagen	bone, skin, soft tissue, serum complement	urine	HPLC, ELISA
Bone sialoprotein (BSP); synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity	bone, dentine, hypertrophic catrilage	serum	RIA, ELISA
Tartarat-resistant acid phosphatase (TR-ACP); osteoclasts, thrombocytes, erythrocytes	bone, blood	plasma/serum	colorimetry, RIA, ELISA
Free gamma carboxyglutamin acid (GLA); resulted from bone proteins (e.g. osteocalcin, matrix Gla protein) and from coagulation factor	blood, bone	serum/urine	HPLC

HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

- Release of neuropeptides (nociceptive and vasoactive) from paradental afferent nerve endings
- Interaction of vasoactive neuropeptides with endothelial cells in strained paradental tissue
- Adhesion of circulated leukocytes to activated endothelial cells
- Migration by diapedesis of leukocytes into the extravascular space

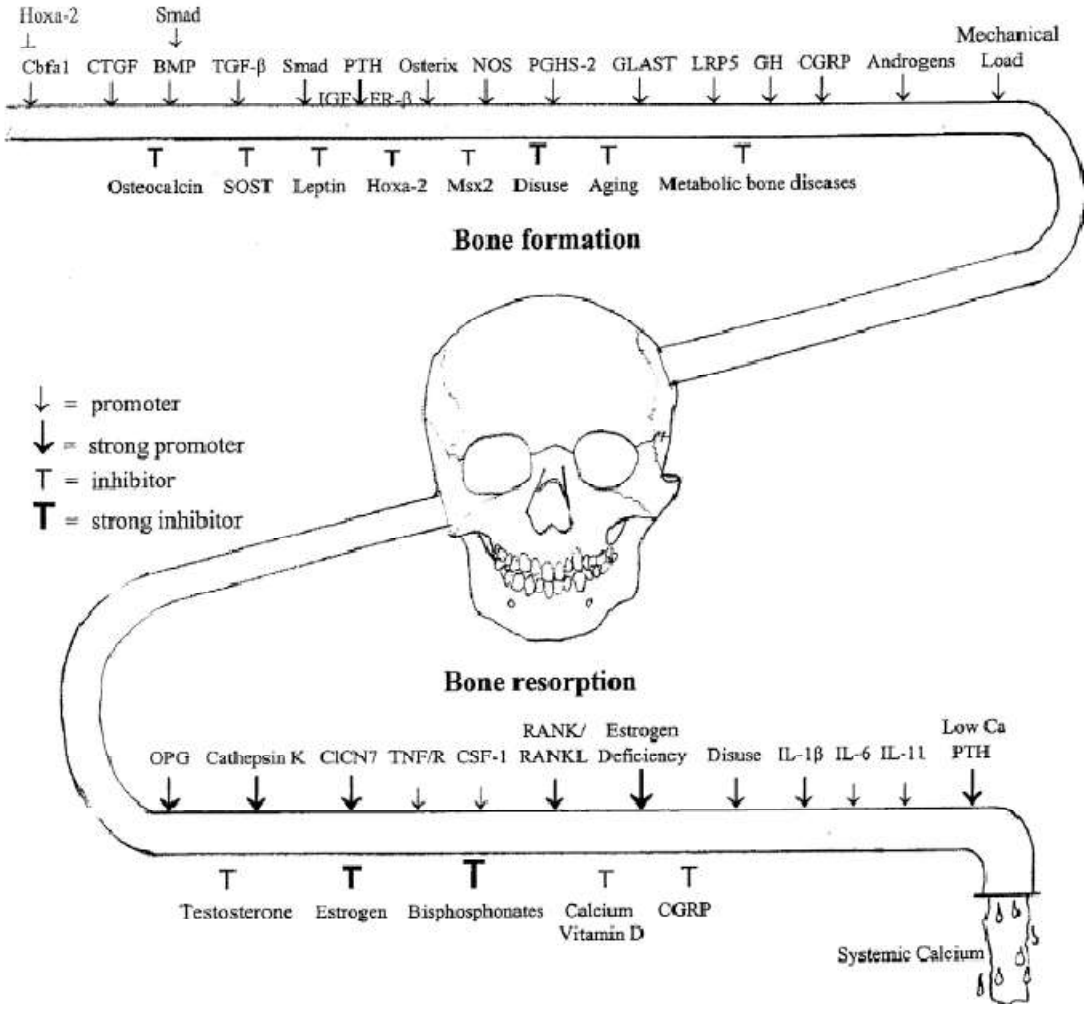

Figure 2. Determinants of skeletal homeostasis and bone changes in OTM. BMP, bone morphogenetic protein; Cbfa1, transcription factor, earliest marker of osteogenesis; CGRP, calcitonin gene-related peptide; ClCN7, chloride channel 7; CSF-1, colony stimulating factor 1; CTGF, connective tissue growth factor; ER-_, estrogen receptor-beta; GH, growth hormone; GLAST, glutamate/aspartate transporter; Hoxa-2, homeobox gene; IGF, insulin-like growth factor; IL-1_, Il-6, Il-11, interleukins; Leptin, central nervous system hormone; LRP5, low-density lipoprotein receptor-related protein 5; Msx-2, homeobox gene; NOS, nitric oxide synthetase; OPG, osteoprotegerin; Osteocalcin, transcription factor; Osterix, transcription factor promoting osteoblast differentiation; PGHS-2, prostaglandin G/H synthetase; PTH, parathyroid hormone; RANK/RANKL, receptor activator of nuclear factor kappa-b and ligand; Smad, cytoplasmic signaling molecules; SOST, gene for sclerostin; TGF-_, transforming growth factor-beta family; TNF/R, tumor necrosis factor and receptor. Taken from Masella RS and Meister M. Current concepts in the biology of orthodontic tooth movement. Am J Orthod and Dentofacial Orthop 2006; 29: 458–468

Table 4. Markers of inflammatory processes. Taken from Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am 2005;49:551-71

Functional group	Increased	Decreased
Complement proteins	C3, C4, C9, Factor B, C1 inhibitor, C4b-binding protein, Mannose-binding lectin	Properdin
Coagulation system proteins	Fibrinogen, Plasminogen, Tissue plasminogen activator, Factor VIII, Urokinase, Protein S, Vitronectin, Plasminogen-activator inhibitor 1	Factor XII
Proteinase inhibitors	Alpha-antitrypsin, Alpha-antichymotrypsin, Inter-alpha-trypsin inhibitors	
Transport proteins	Ceruloplasmin Haptoglobin, Haemopexin, Ferritin	Transferin, Transthyretin, Thyroxin-binding globulin
Others	C-RP, Serum amyloid A, Secreted phospholipase A, Lipopolysaccharide-binding protein, Interleukin-1-binding protein, Interleukin-1 receptor antagonist, Granulocyte colony stimulating factor, Alpha-acid glycoprotein, Fibronectin, Angiotensinogen	Albumin, Alpha-fetoprotein, Insulin-like growth factor

Table 5. Markers involved in orthodontic tooth movement collected from dental tissues (alveolar bone, periodontium and pulp).Taken from Cellular and Molecular Changes in Orthodontic Tooth Movement. TheScienti cWorldJOURNAL 2011; 11: 1788–1803

Marker	Function	Sample	Method	Sources
Bone morphogenetic proteins (BMPs)	Bone formation	Rat pulp tissue	Quantitative RT-PCR	[7, 8]
Cathepsin K	Root and bone resorption; expressed in odontoclasts and osteoclasts	Rat maxillary bone	Hybridization	[9]
Endothelin-1, endothelin receptors (ET_A and ET_B)	Stimulates the proliferation of osteoblasts; their downregulation indicates the end of stage 2 and start of stage 3	Rat alveolar bone	RT-PCR	[10]
Endothelial nitric oxide synthase (eNOS)	Mediates bone formation in the tension area	Rat maxilla tissue	Immunohistochemistry	[11]
Inducible nitric oxide synthase (iNOS)	Mediates inflammation-induced bone resorption in the compression area.	Rat maxilla tissue	Immunohistochemistry	[11]
Ki-67	Proliferation	Rat maxilla	Immunohistochemistry	[12]
Muscle segment homeobox 1 (Msx1)	Regulator for bone formation	Human alveolar mucoperiosteum	Quantitative RT-PCR, Immunohistochemistry	[7, 13]
Muscle segment homeobox 2 (Msx2)	Regulator for bone formation	Mouse periodontal ligament tissue	Histopathological, Immunohistochemistry	[7, 14]
Osteoprotegerin (OPG)	Osteoclastogenesis-inhibitory factor	Rat mandible	RT-PCR	[15]
Receptor activator of nuclear factor kappa B ligand (RANKL)	Osteoclastic differentiation	Rat maxilla	Immunohistochemistry	[12]
Runx2	Osteoblast precursor	Rat maxilla	Immunohistochemistry	[12]

Table 6. Markers involved in orthodontic tooth movement collected from serum.Taken from Cellular and Molecular Changes in Orthodontic Tooth Movement. TheScienti cWorldJOURNAL 2011; 11: 1788–1803

Marker	Function	Sample	Method	Sources
N-terminal propeptide of type 1 procollagen	Bone turnover	Serum of premenopausal woman	ELISA	[16]
Osteocalcin	Bone turnover	Serum of premenopausal woman	ELISA	[16]
C-telopeptide of type 1 collagen (CTX)	Bone turnover	Serum of premenopausal woman	ELISA	[16]
Insulin-like growth factor-1	Decreases collagen degradation, increases bone matrix deposition, and recruits osteoblasts	Serum of premenopausal woman	ELISA	[16]

- Synthesis and release of signalling molecules by leukocytes that have migrated into the strained paradental tissues
- Interaction of various types of paradental cells with the signal molecules released by the migratory leukocytes
- Activation of the cells to participate in the modelling and remodelling of the paradental tissues

Biomarker

A biomarker is a substance that is measured and evaluated objectively as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Taba et al., 2005). A biomarker should be specific and sensitive and have the ability to inform about the biological condition in terms of periodontal tissue changes and their relationships with the particular phase of OTM.

Knowledge about the type of cellular process will give a good idea of giving proper mechanical loading and thus shorten the period of treatment, which can also aid in avoiding adverse effects associated with orthodontic treatment. The biomarkers involved in various biological processes are listed as follows:

- Markers of Alveolar Bone Remodelling[4]
- Bone Formation Markers
- Bone Resorption Markers

Markers of Root Resorption (Dolce et al., 2002)

Root resorption is either a physiological or pathological condition associated with tooth structure loss and is caused by osteoclastic cells.

Table 7. Markers involved in orthodontic tooth movement collected from gingival cerivular fluid and saliva. Taken from Cellular and Molecular Changes in Orthodontic Tooth Movement. TheScienti cWorldJOURNAL 2011; 11: 1788–1803

Marker	Function	Sample	Method	Sources
Alkaline phosphatase (ALP)	Bone formation	Human saliva and GCF	Enzyme assay	[17–19]
Aspartate aminotransferase (AST)	Tissue damage and inflammatory process	Human GCF	Enzyme assay	[20–22]
Cathepsin B	Resolution organic matrix; bone resorption	Human GCF	Fluorometry, enzyme assay, Western blot	[23, 24]
Dentine phosphoprotein (DPP)	Root resorption	Human GCF	ELISA	[25]
Dentine sialoprotein (DSP)	Root resorption	Human GCF	Western blot	[26]
Activity index of Interleukin-1β and interleukin-1 receptor antagonist	IL-1β is potent for bone resorption and the inhibition of bone formation	Human GCF	ELISA	[27]
Interleukin-2 (IL-2)	B-cell activation, stimulates macrophages and NK Cell, T-cell proliferation, osteoclastic activity	Human GCF	Immunoassay	[28]
Interleukin-6 (IL-6)	Stimulates osteoclast formation and bone resorbing activity of preformed osteoclasts	Human GCF	Immunoassay	[28]
Interleukin-8 (IL-8)	Recruitment and activation of neutrophils	Human GCF	Immunoassay	[28]
Lactate dehydrogenase (LDH)	For monitoring periodontal metabolic changes, index of tissue destruction	Human saliva and GCF	Enzyme assay	[19, 29, 30]
Matrix metalloproteinase-1 (MMP-1)	PDL remodelling during initial tooth movement	Human GCF	Western blot	[31]
Matrix metalloproteinase-2 (MMP-2)	PDL remodelling during initial tooth movement	Human GCF	Western blot	[31]
Myeloperoxidase	To asses inflammation in orthodontic movement	Human GCF	Enzyme assay	[32]
Tartrate resistant acid phosphatase (TRAP)	Osteoclastic differentiation	Human GCF	Enzyme assay	[19]

Orthodontic treatment invariably results in permanent root resorption (Estrela et al., 2009). Dentin matrix protein 1 (DMP1), dentin phosphophoryn (PP), dentin sialoprotein (DSPP), IL-1β, IL-6, and TNF-α are the markers of root resorption. Early detection of root resorption during orthodontic treatment is essential for identifying teeth at risk of severe resorption. At present, detection of root resorption is obtained using radiographic techniques which are technique sensitive require radiation exposure.

Markers of In ammatory Processes

- Markers involved in orthodontic tooth movement collected from dental tissues (Shahrul Hisham Zainal Arif n et al., 2011) (alveolar bone, periodontium and pulp).
- Markers involved in orthodontic tooth movement collected from serum (Shahrul Hisham Zainal Arif n et al., 2011).
- Markers involved in orthodontic tooth movement collected from Gingival cervicular fluid and saliva (Shahrul Hisham Zainal Arif n et al., 2011).

Clinical implications

Remodeling changes in paradental tissues are considered essential in effecting orthodontic tooth movement. The force-induced tissue strain produces local alterations in vascularity, as well as cellular and extracellular matrix reorganization, leading to the synthesis and release of various neurotransmitters, cytokines, growth factors, colony-stimulating factors, and metabolites of arachidonic acid.[8] Biomarkers have a significant role in tooth movement. Potential biological markers can be collected from different tissue samples and suitable sampling is important to accurately reflect biological processes. A thorough knowledge about the biomarkers in orthodontic tooth movement and their mechanism of action should provide a rationale for better orthodontic treatment.

Conclusion

Biomarkers discussed in this review help in providing a better understanding of the ongoing cellular process during orthodontic treatment.

The development of biomarkers will go on and will keep providing vital information of the microenvironment. Thus, the knowledge of all the biomarkers present in the GCF that can be used to mark the changes in tooth that is undergoing orthodontic treatment may be of clinical usefulness leading to proper choice of mechanical loading, to improve and to shorten the period of treatment, avoiding adverse consequences Several possible biomarkers representing the biological changes during specific phenomenon, that is, bone remodelling (formation and resorption), inflammation, and root resorption have also been listed. The knowledge of these biomarkers could be used in accelerating orthodontic treatment

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