Published in final edited form as:

J Dent Res. 2008 May ; 87(5): 414–434.

Mechanisms of Tooth Eruption and Orthodontic Tooth Movement

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Abstract

Teeth move through alveolar bone, whether through the normal process of tooth eruption or by strains generated by orthodontic appliances. Both eruption and orthodontics accomplish this feat through similar fundamental biological processes, osteoclastogenesis and osteogenesis, but there are differences that make their mechanisms unique. A better appreciation of the molecular and cellular events that regulate osteoclastogenesis and osteogenesis in eruption and orthodontics is not only central to our understanding of how these processes occur, but also is needed for ultimate development of the means to control them. Possible future studies in these areas are also discussed, with particular emphasis on translation of fundamental knowledge to improve dental treatments.

Keywords

dental follicle; periodontal ligament; osteoclastogenesis; osteogenesis; RANKL; OPG; CSF-1; bone remodeling; bone formation; bone resorption

INTRODUCTION

Given the breadth of the two topics, tooth eruption and orthodontic tooth movement, this review will focus more upon what is currently known about their molecular mechanisms, commonalities, and differences, instead of a lengthy historical review. For the latter, readers are referred to other reviews (Cahill *et al.*, 1988; Marks and Schroeder, 1996; Wise *et al.*, 2002; Krishnan and Davidovitch, 2006; Masella and Meister, 2006; Meikle, 2006).

Theories of Tooth Eruption

For the past 70 years, various theories have been presented on the mechanisms of tooth eruption. That numerous theories abound may be due to the enormous success of orthodontics in moving teeth with force application. However, tooth eruption is a fundamental developmental and physiologic process, and force plays a secondary role. Regardless, some of these theories are discussed in the section of this review entitled “Motive Force of Tooth Eruption”. Previous reviews in the past 20 years also have considered the various theories of eruption (*e.g.*, see Cahill *et al.*, 1988; Marks and Schroeder, 1996; Wise *et al.*, 2002).

In this review, emphasis will be on the dental follicle and its role in initiating eruption by regulating alveolar bone resorption and alveolar bone formation. This focus emanates from the pioneering work of Sandy Marks, Jr., and Don Cahill, who demonstrated that the dental follicle was required for eruption. Their surgical studies utilizing dog premolars showed that removal of the follicle from the unerupted tooth prevented the tooth from erupting (Cahill and Marks,

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1980), whereas leaving the follicle intact and substituting an inert object for the tooth resulted in eruption of the inert object (Marks and Cahill, 1984).

One caveat to remember in this review is that we are focusing on teeth of limited eruption (*e.g.*, human dentition, rodent molars), not on teeth of continuous eruption (*e.g.*, rodent incisors). Different molecules and mechanisms appear to regulate eruption in the two types of teeth. Regarding the molecules, epidermal growth factor accelerates the time of eruption in rodent incisors, but has little effect on the molars (Lin *et al.*, 1992; Cielinski *et al.*, 1995), whereas colony-stimulating factor-1 (CSF-1) accelerates rat molar eruption, but not incisor eruption (Cielinski *et al.*, 1994, 1995). Injection of dexamethasone also has contrasting effects, in that it accelerates incisor eruption, but not molar eruption (Wise *et al.*, 2001).

Pathophysiology of Orthodontic Tooth Movement

Unlike tooth eruption, orthodontic tooth movement is a process that combines both pathologic and physiologic responses to externally applied forces. With the possible exception of tooth drift, which in some ways resembles eruption (King *et al.*, 1991a), orthodontic tooth movement is accompanied by minor reversible injury to the tooth-supporting tissues. Superimposed on this is the physiologic adaptation of alveolar bone to mechanical strains. Therefore, relevant inflammatory mechanisms need to be considered along with skeletal mechanotransduction for a full understanding of orthodontic tooth movement. The evidence for injury and its resolution in orthodontic tooth movement will be considered in this section, and theories for skeletal mechanotransduction will be reviewed separately in a later section. Despite these differences, one similarity in both orthodontic and tooth eruption movement is the requirement for an intervening biologically active soft tissue. In the case of eruption, this is the dental follicle, while in orthodontics it is the periodontal ligament. The data supporting evidence for both of these requirements will be considered in the following section.

The clinical picture of orthodontic tooth movement consists of three phases: an initial and almost instantaneous tooth displacement; delay, where no visible movement occurs; and a period of linear tooth movement. The applied forces create strains in the tooth-supporting tissues that manifest almost immediately and can be roughly categorized as compressive and tensile. In the absence of transducer data directly measuring these strains, various finite element models have been created to describe them. Finite element analyses of the load transfer from the tooth through the periodontal ligament to the alveolar bone must account for the physical properties and morphology of the periodontium. The periodontal ligament is known to be a non-linear visco-elastic material, but orthodontic finite element models often incorporate homogeneous, linear elastic, isotropic, and continuous periodontal ligament properties. Also, adjustments for differences in micromorphology have not been made. Finite element studies that attempt to account for these report that loading of the periodontium cannot be explained in simple terms of compression and tension along the loading direction. Also, tension seems to be far more common than compression (Cattaneo *et al.*, 2005). However, because the pressure-tension terminology is so prevalent in the literature, and it generally can serve as a convenient means to distinguish the different processes accompanying orthodontic tooth movement, it will be used here.

The initiating inflammatory event at compression sites is caused by constriction of the periodontal ligament microvasculature, resulting in a focal necrosis, known by its histological appearance as hyalinization, and compensatory hyperemia in the adjacent periodontal ligament (Murrell *et al.*, 1996) and pulpal vessels (Kvinnsland *et al.*, 1989). These necrotic sites release various chemo-attractants (Lindskog and Lilja, 1983) that draw giant, phagocytic, multi-nucleated, tartrate-resistant acid-phos phatase-positive cells to the periphery of the necrotic periodontal ligament (Brudvik and Rygh, 1994a,b). These cells resorb the necrotic periodontal ligament, as well as the underlying bone and cementum (Fig. 1A). Osteoclasts are recruited

from the adjacent marrow spaces (Rody *et al.*, 2001). Until these cells can be recruited and the necrosis removed, tooth movement is impeded, resulting in the clinical manifestation of a delay period. This is followed by deposition of new cementum (Brudvik and Rygh, 1995; Casa *et al.*, 2006), pulpal secondary dentin (Nixon *et al.*, 1993), and bone (King *et al.*, 1991b) in the vicinity of resorption sites.

There is abundant evidence suggesting that neurovascular mechanisms play important roles in tooth movement, through the development of an inflammatory reaction. Elevations in the periodontal ligament neurotransmitters, CGRP (Kvinnsland and Kvinnsland, 1990) and substance P (Nicolay *et al.*, 1990), can persist for extended periods following orthodontic tooth movement (Norevall *et al.*, 1995, 1998). Moreover, these have the ability to cause vasodilatation and increased vessel permeability, accompanied by the proliferation of endothelial cells and fibroblasts (Hall *et al.*, 2001), as well as the extravasation of leukocytes (Toms *et al.*, 2000). The distribution and intensity of immunoreactive staining for other bioactive factors associated with periodontal ligament nerves (Saito *et al.*, 1993; Deguchi *et al.*, 2003) and endothelium (Lew *et al.*, 1989; Lew, 1989; Sims, 1999; Drevensek *et al.*, 2006) also correlate well with either mechanically induced tissue remodeling responses or enhanced orthodontic tooth movement. Also, severing the inferior alveolar nerve postpones increased periodontal ligament blood flow following force application (Vandevska-Radunovic *et al.*, 1998).

The release of pro-inflammatory cytokines and lysosomal enzymes that promote tissue resorption at compression sites is well-documented. Prostaglandins, IL-1, IL-6, TNF α , and receptor activator of nuclear factor kappa B ligand (RANKL) are all elevated in the periodontal ligament during tooth movement (Yamaguchi and Kasai, 2005). Increases in the lysosomal enzymes, acid phosphatase, tartrate-resistant acid phosphatase (Lilja *et al.*, 1983, 1984; Keeling *et al.*, 1993), and cathepsin B (Yamaguchi *et al.*, 2004) are also localized at compression sites, suggesting that they may play pivotal roles during orthodontic tooth movement in the process of hard- and soft-tissue degradation by increased numbers of macrophage and dendritic-like cells (Vandevska-Radunovic *et al.*, 1997).

Tension sites in orthodontic samples generally have been characterized as being primarily osteogenic, without a significant inflammatory component (Fig. 1B). However, there is evidence that inflammatory responses to tension may be strain-dependent, since tensile strains of low magnitude are anti-inflammatory and induce magnitude-dependent anabolic signals in osteoblast-like periodontal ligament cells, culminating in the regulation of inflammatory gene transcription (Long *et al.*, 2001). In contrast, high tensile strains act as pro-inflammatory stimuli and increase the expression of inflammatory cytokines (Long *et al.*, 2002). This finding has recently been confirmed in a tooth movement model where sites presumed to be in low tensile strain exhibited a marked absence of IL-1 α and COX-2, while those presumed to be compressive or having high tensile strains showed up-regulation of IL-1 α and COX-2 (Madhavan *et al.*, 2008, submitted). Morphological evidence of periodontal ligament cellular disruption at tension sites in tooth movement also has been described after only 5 min of loading, further suggesting the involvement of an inflammatory mechanism (Orellana *et al.*, 2002; Orellana-Lezcano *et al.*, 2005). Despite this, the mechanism for osteogenesis at tension sites in tooth movement is not well-understood, but reasonable inferences can be made from various mechanotransduction models. These will be discussed in a subsequent section of this review.

One issue that, at first, seems paradoxical is the observation that compression sites in orthodontic tooth movement are primarily resorptive, while the tensile sites are osteogenic. This seems contrary to the bone mechanical usage literature, which describes loaded sites as being osteogenic and unloaded sites as resorptive (Frost, 2004). There are two possible

explanations for these differences. First, compression sites clearly have a tissue injury component superimposed on physiological transduction, with the former producing inflammatory products that are primarily resorptive and stimulate cells to remove the injured tissue. Second, resorption at compression sites in tooth movement could be perceived as a result of lowering of the normal strain from the functioning periodontal ligament, while osteogenesis at tension sites could be a reflection of loading of the principal fibers of the periodontal ligament (Melsen, 2001). The latter could also be accompanied by strains in the alveolar process transmitted either through the principal fibers of the periodontal ligament or by direct impingement of the tooth root on the alveolar bone.

Another important distinction between orthodontic tooth movement and eruption may be that there is considerable variation in the response of periodontal ligament tissues to tooth movement. This can be due not only to differences in biomechanical signals, but also to specific host differences, such as diurnal rhythms (Miyoshi *et al.*, 2001), occlusion (Esashika *et al.*, 2003), systemic metabolism (Verna and Melsen, 2003), age (King *et al.*, 1995; Kyomen and Tanne, 1997; Ren *et al.*, 2003), or normal variation in bony trabeculation.

REQUIREMENTS FOR TOOTH ERUPTION AND ORTHODONTIC TOOTH MOVEMENT

There are two fundamental requirements for both tooth eruption and orthodontic tooth movement to occur: (1) Both require soft tissue, intervening between tooth structure and alveolar bone, which plays an important role in regulating the remodeling of adjacent tissues; and (2) both require bone turnover that is temporally and spatially regulated to facilitate specific translocations of teeth through alveolar bone.

The Intervening Biologically Active Soft Tissues

Dental Follicle for Eruption—With the publication of their seminal papers on the necessity of the dental follicle for tooth eruption, Sandy Marks and Don Cahill not only altered our views of tooth eruption, but also gave us a tissue on which to focus as the molecular regulator of eruption. Interposed between the alveolar bone of the socket and the enamel organ of the unerupted tooth, the dental follicle is a loose connective tissue sac that is ideally positioned to regulate alveolar bone activity (see Fig. 2A). It is highly likely that the reason the dental follicle is needed for eruption is because it initiates and regulates the required osteoclastogenesis and osteogenesis, at least for the intraosseous phase of eruption leading to tooth emergence. For the supra-osseous phase of eruption, in which the tooth moves to its final occlusal plane, the follicle may play a lesser role, while biomechanical influences may become more important. As demonstrated by Cahill and Marks (1982) in the dog premolar, not until the supra-osseous phase is the dental follicle attached to the alveolar bone and cementum, becoming the periodontal ligament. Similarly, in the first mandibular molar of the rat, the dental follicle becomes the periodontal ligament (Fig. 2B) and does not attach to the alveolar bone and cementum until approximately day 18—the emergence time of the tooth (Wise *et al.*, 2007). In turn, this periodontal ligament could aid in moving the tooth to its occlusal plane during the supra-osseous phase, not unlike what appears to occur in the continuously growing incisors of the rodent (Moxham and Berkovitz, 1974) or rabbit (Berkovitz and Thomas, 1969).

As a side issue, it should be noted that stem cells have been shown to be present in the periodontal ligament (Seo *et al.*, 2004; Nagatomo *et al.*, 2006; Jo *et al.*, 2007; Techawattanawisal *et al.*, 2007) and in the dental follicle (Yao and Wise, unpublished observations). In the dental follicle, we have shown that these stem cells are pluripotent and capable of differentiating into other cell types, such as adipocytes, neurons, and osteoblasts.

Thus, the possibility exists that such stem cells could contribute to alveolar bone formation, as well as form cementoblasts. Their role in tooth eruption, if any, is unknown.

Periodontal Ligament for Tooth Movement—The examples of tooth ankylosis and implants serve to demonstrate the essential role of the periodontal ligament in tooth movement. Ankylosed teeth have focal lesions characterized by bony bridges that eliminate the periodontal ligament in these areas. Similarly, implants with or without osseointegration also lack a periodontal ligament. In both instances, teeth are unresponsive to orthodontic tooth movement and dental drift. An appreciation of this has provided the impetus for the recent wide acceptance by orthodontists of mini-implants as temporary anchorage devices. It also explains why ankylosed deciduous teeth appear to submerge as adjacent teeth continue to adjust to vertical facial growth.

The specific underlying role of the periodontal ligament in tooth movement is not well-understood, but its unique biomechanical, cellular, and molecular natures are undoubtedly important. From a biomaterials perspective, the periodontal ligament is a complex, fiber-reinforced substance that responds to force in a viscoelastic and non-linear manner (Jonsdottir *et al.*, 2006). This response is characterized by an instantaneous displacement, followed by a more gradual (creep) displacement that reaches a maximum after 5 hrs (van Driel *et al.*, 2000), suggesting that fluid compartments within the periodontal ligament may play an important role in the transmission and damping of forces acting on teeth. The strains that are created in the periodontal ligament by force application clearly have biological consequences for the tissue itself, and possibly also for the other tooth-supporting tissues (*i.e.*, alveolar bone and cementum).

Periodontal ligament cells respond to force by increases in cell proliferation and apoptosis. The relative extent to which these two competing processes occur controls the various cell populations in the periodontal ligament and reflects the specific biomechanics (Mabuchi *et al.*, 2002).

The major fibrous components of the periodontal ligament extracellular matrix (collagen, tropoelastin, and fibronectin) also show enhanced expression following force application (Howard *et al.*, 1998; Redlich *et al.*, 2004a). Matrix metalloproteinases (MMPs) and their specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs), act in a coordinated fashion to regulate collagen remodeling. The periodontal ligament expression levels of MMP-2, 8, 9, 13, and TIMPs 1-3 increase transiently during orthodontic tooth movement. However, these genes have different patterns of expression at compression and tension sites, suggesting that collagen remodeling is regulated differentially based on mechanics (Howard *et al.*, 1998; Takahashi *et al.*, 2003, 2006). This conclusion is given further support by the observations that tension prevents degradation of the matrix by inhibiting MMP-1 (Arnoczky *et al.*, 2004), while relaxation of tension enhances extracellular matrix resorption (Von den Hoff, 2003). Enhanced expression of MMP-1 in periodontal ligament fibroblasts also may be the result of a direct effect of force on the gene (Redlich *et al.*, 2004b).

Matrix proteoglycans are also altered in the periodontal ligament during orthodontic tooth movement. Periodontal ligament chondroitin sulfate (CS) and heparin sulfate (HS) increase during tooth movement and decrease in hypofunction. However, the complex patterns of CS and HS changes in tooth movement make interpretation of their roles difficult (Esashika *et al.*, 2003). Hyaluronic acid (HA), present in the periodontal ligament, may bind with increased amounts of versican, a large HA-binding proteoglycan, and link protein localized at compressive sites, to create large hydrated aggregates. These may act either to limit tissue damage by dissipating excessive compressive forces, or to provide space to facilitate the migration of resorptive cells into these sites (Sato *et al.*, 2002).

The Turnover of Adjacent Alveolar Bone

Frost's pioneering descriptions of how bone turns over have provided researchers and clinicians with important concepts that have improved our understanding of numerous bony processes that were previously viewed as unrelated, including osteoporosis, fracture healing, mechanical usage, metabolic and genetic bone disease, and skeletal growth and development (Frost, 2001). These concepts can also provide important insights into the differences and similarities between dental eruption and orthodontics.

Bones turn over by two related, but distinct, processes Frost called 'modeling' and 'remodeling'. Modeling is characterized by either osteogenesis or resorption that is sustained over a specific period of time and at precise bony surfaces. It results in skeletal shape change and translocation of hard-tissue structures. Modeling processes are prevalent in skeletal development, where individual bones move in relation to each other and change shape. The intra-osseous phase of tooth eruption can be considered to be primarily a process of alveolar bone modeling.

Bone Resorption (Modeling) in Eruption—Given that the unerupted tooth is encased in alveolar bone, bone resorption is required for the tooth to erupt. In turn, osteoclast formation (osteoclastogenesis) is needed for an adequate number of osteoclasts to be present to resorb the alveolar bone.

A unique feature of bone resorption in the formation of an eruption pathway is that it can be uncoupled from tooth eruption—*i.e.*, the tooth does not have to move for the eruption pathway to form. This observation lends support to the idea that the bone modeling in tooth eruption is genetically controlled, and not mechanically regulated by the eruption of the tooth.

Immobilizing the erupting permanent third premolars in the mandible in the dog does not stop the formation of the eruption pathway (Cahill, 1969a). The osteoclasts resorbing the alveolar bone appear to arise from an influx of mononuclear cells (osteoclast precursors) into the dental follicle at a specific time prior to eruption, as shown in the dog (Marks *et al.*, 1983), rat (Wise and Fan, 1989), and mouse (Volejnikova *et al.*, 1997). In turn, osteoclast numbers increase on the alveolar bone surface at the same time as a result of fusion of these mononuclear cells to form osteoclasts (Marks *et al.*, 1983; Wise *et al.*, 1985).

At the ultrastructural level, the architecture of the alveolar bone reveals that bone resorption is occurring in the coronal region of the bony crypt prior to and during the intra-osseous phase of eruption. Specifically, in the dog, the architecture of the bone in the coronal region of the crypt appears scalloped (Marks and Cahill, 1986), a finding confirmed for the socket of the first mandibular molar of the rat (Wise *et al.*, 2007).

Numerous experiments have confirmed that bone resorption is required for tooth eruption. Injection into rats of a bisphosphonate, pamidronate, which slows resorption, results in a delay in the time of molar eruption (Grier and Wise, 1998). Bafilomycin A₂, another agent that inhibits osteoclast activity, has also been reported to inhibit tooth eruption (Sundquist and Marks, 1994), although some of the toxic effects of this molecule may also affect eruption. Conversely, injecting colony-stimulating factor-1, a molecule that promotes osteoclastogenesis, accelerates the time of eruption (Cielinski *et al.*, 1994, 1995).

Inhibition of the molecules that promote osteoclastogenesis can inhibit eruption. In knock-out mice devoid of receptor activator of nuclear factor kappa B (RANKL), the teeth do not erupt (Kong *et al.*, 1999). In osteopetrotic rodents in which osteoclasts are either absent or non-functional, teeth do not erupt. Molecular analyses have shown that osteopetrotic mice (op/op) do not have functional colony-stimulating factor-1 (Felix *et al.*, 1990; Wiktor-Jedrzejczak *et al.*, 1990; Yoshida *et al.*, 1990), and the osteopetrotic toothless (*tl*) rat is the result of a loss-

of-function frameshift mutation in the *CSF-1* gene (Dobbins *et al.*, 2002; Van Wesenbeeck *et al.*, 2002). Injection of CSF-1 into these animals at an early age prior to the onset of eruption will induce eruption (Iizuka *et al.*, 1992).

Bone Remodeling—Bone remodeling is a cyclic process that is a response to the need for continuous repair and renewal of the skeleton throughout life. Frost describes a basic multicellular unit that performs a coordinated series of events comprising the remodeling cycle. A remodeling cycle has four phases: activation, resorption, reversal, and formation. Although this sequence of events has been confirmed in numerous contexts and is widely accepted as the way that the skeleton repairs itself, the precise mechanisms controlling the basic multicellular units are not well-understood. The timing of the histological events occurring at compression sites in orthodontic tooth movement is consistent with a remodeling cycle (King *et al.*, 1991b) (Fig. 2B). This, along with the abundant evidence for tissue damage at these sites, strongly suggests that remodeling is a prevalent bone turnover process at orthodontic compression sites.

One important consideration is how remodeling cycles are initiated. Much experimental evidence has linked bone remodeling to microdamage, and to subsequent increased cellular activity. Microcracks in bone caused by fatigue or trauma may play an important role in the initiation of remodeling cycles (Galley *et al.*, 2006), because crack displacements are capable of tearing osteocyte cell processes, which may directly secrete bioactive molecules into the extracellular matrix, triggering a response (Hazenber *et al.*, 2006). The increased prevalence of microcracks at compression sites in orthodontic tooth movement further suggests that they are important in initiating orthodontic bone remodeling (Verna *et al.*, 2004).

Another important bone remodeling concept is coupling between resorption and formation. Coupling mechanisms have been postulated as a means by which bone is neither lost nor gained during repair. The exact mechanism by which coupling is achieved is not well-understood, but is thought to be controlled by the release of paracrine molecules by the cells of the basic multicellular unit. During the early stages of repair in tooth movement, the occurrence of several paracrine factors (*e.g.*, IGF-II, IGFBP-5 or -6) within lacunae and in cementoblasts suggests that these may be involved in controlling this remodeling sequence (Hazenber *et al.*, 2006).

Another related coupling issue involves the relative rates of resorption *vs.* formation. The former is quite rapid, while the latter is significantly slower. This has important consequences for bones that are undergoing extensive remodeling—for example, during the perimenopausal period (Recker *et al.*, 2004). In these instances, bone formation cannot keep pace with the large amounts of resorption that are occurring, with the end result being net bone loss. The failure of formation to keep up with resorption during the extensive amount of remodeling at compression sites during orthodontic treatment also may explain the common clinical finding of tooth mobility and widening of the periodontal ligament space.

Alveolar bone resorption and formation appear not to be coupled in eruption—*i.e.*, resorption occurs in the coronal portion of the alveolar bony crypt (socket), whereas bone formation occurs at the base of the socket. Moreover, if one process, such as bone formation, is blocked by temporarily impacting the erupting tooth, bone resorption continues, and an eruption pathway is formed (Cahill, 1969a). However, given that bone formation occurs rapidly at the base of the socket once the restraint on the erupting tooth is removed (Cahill, 1969b), one cannot fully eliminate the possibility that communication between the basal and coronal halves of the bony socket may occur and, in turn, perhaps influence the rate of bone formation or resorption occurring in the basal and coronal halves, respectively.

MOLECULAR REGULATION OF OSTEOCLASTOGENESIS

Background

Osteoclast biology underwent a revolution in the late 1990s, not only with the discovery of a critical set of molecules that regulated osteoclastogenesis, but also with the elucidation of how they interacted. In particular, a member of the TNF ligand family, RANKL, was initially found to be a membrane-bound protein present on osteoblasts and stromal cells, as well as on other cell types (Anderson *et al.*, 1997; Wong *et al.*, 1997; Yasuda *et al.*, 1998a). Cell-to-cell signaling between cells with RANKL on their surfaces and osteoclast precursors carrying the receptor RANK induced both osteoclast formation and activation (Yasuda *et al.*, 1999). Three isoforms of RANKL have since been identified, two of which are transmembrane proteins, whereas the third—RANKL3—is a soluble form (Ikeda *et al.*, 2001).

The receptor for RANKL on osteoclast precursors is the receptor activator of NF- κ B (RANK), first identified by Anderson *et al.* (1997). In turn, CSF-1 is required to up-regulate RANK gene expression in osteoclast precursors (Arai *et al.*, 1999), and this is one of the reasons that CSF-1 is required for osteoclastogenesis. The growth and differentiation of mononuclear pre-osteoclasts are also dependent upon CSF-1 (Stanley *et al.*, 1983; Tanaka *et al.*, 1993). Moreover, CSF-1 appears to have chemotactic properties for recruiting osteoclast progenitors (Wang *et al.*, 1988; Bober *et al.*, 1995; Que and Wise, 1997).

The cell-to-cell signaling involving the binding of RANKL to RANK results in recruitment of various members of TNF receptor-associated factors (TRAFs) within the osteoclast precursor, of which TRAF6 appears to be a key player (Darnay *et al.*, 1999; Wong *et al.*, 1999). For example, TRAF6 activates the signaling pathways for NF κ B and c-Fos (Boyle *et al.*, 2003). Null mice devoid of the *c-Fos* gene have no osteoclasts, but they do have osteoclast precursors (Grigoriadis *et al.*, 1994), and the same is true for mice devoid of the NF κ B genes (Franzoso *et al.*, 1997; Iotsova *et al.*, 1997). Moreover, in these null mice, the teeth do not erupt. Of particular interest regarding c-Fos is that RANKL signaling through c-Fos induces the interferon- β (IFN- β) gene in osteoclast progenitor cells, and the IFN- β synthesized negatively feeds back on the cells to inhibit the expression of c-Fos (Takayanagi *et al.*, 2002).

TRAF6 also binds Src tyrosine kinase, which likely is the effector molecule in osteoclast activation, because it is required for cytoskeletal protein re-arrangement to form a ruffled border (Boyce *et al.*, 1992). Src also appears to promote osteoclast survival by preventing apoptosis (Wong *et al.*, 1999; Xing *et al.*, 2001).

A means of either fine-tuning or inhibiting the stimulation of osteoclastogenesis is obviously needed. The molecule that does this is osteoprotegerin, a secreted glycoprotein that is a decoy-receptor for RANKL (Simonet *et al.*, 1997; Tsuda *et al.*, 1997; Yasuda *et al.*, 1998b). Binding of osteoprotegerin to RANKL inhibits the cell-to-cell signaling that occurs between cells with RANKL on their membrane and osteoclast precursors, resulting in the inhibition of osteoclastogenesis (Yasuda *et al.*, 1998b, 1999). *In vivo*, over-expression of osteoprotegerin in transgenic mice results in osteopetrosis and few osteoclasts, although TRAP-positive mononuclear cells (pre-osteoclasts) are present (Simonet *et al.*, 1997). Injection of recombinant osteoprotegerin into mice also produces the same results (Simonet *et al.*, 1997).

Fusion of the osteoclast precursors to form osteoclasts appears to require a transmembrane receptor molecule, dendritic cell-specific transmembrane protein (DC-STAMP) (Kukita *et al.*, 2004; Yagi *et al.*, 2005). Gene expression of DC-STAMP is induced in osteoclast precursors by RANKL, and inhibition of this expression by small interfering RNAs inhibits osteoclast formation (Kukita *et al.*, 2004). DC-STAMP knock-out mice also have no multi-nucleated

osteoclasts, but do have mononuclear cells that are tartrate-resistant acid-phosphatase (TRAP)-positive (Yagi *et al.*, 2005).

Another molecule that may affect fusion is secreted frizzled-related protein-1 (SFRP-1), a molecule that inhibits osteoclastogenesis (Häusler *et al.*, 2004). This molecule also is secreted by the dental follicle, and *in vitro* osteoclastogenic assays show that, although it prevents osteoclast formation, increasing concentrations of SFRP-1 result in increased numbers of TRAP-positive mononuclear cells (Liu and Wise, 2007). Thus, SFRP-1 may act to prevent osteoclastogenesis by preventing fusion of the precursor cells. Molecules involved in osteoclastogenesis can be found in Table 1.

In Tooth Eruption

With the knowledge accumulated as to what is required for osteoclastogenesis at the molecular level, how is osteoclastogenesis regulated for tooth eruption? An early clue was provided with the findings that, for teeth of limited eruption (which includes human dentition), the times of osteoclastogenesis are well-defined. In effect, there is a major burst of osteoclastogenesis that occurs before the onset of eruption, and a minor burst that occurs after eruption begins. For the rat mandibular first molar, the major burst of osteoclastogenesis, with the maximal number of osteoclasts on alveolar bone and osteoclast precursors in the dental follicle, is seen at day 3 post-natally, with the minor burst at day 10 (Cielinski *et al.*, 1994; Wise and Fan, 1989); for the mouse first mandibular molar, the major burst is at day 5 post-natally, with the minor at day 9 (Volejnikova *et al.*, 1997); and for the dog 3rd and 4th premolars, the major burst is at week 16, with the minor (osteoclasts only) at week 20 (Marks *et al.*, 1983).

Given that the dental follicle is required for eruption, and that osteoclast precursors are being recruited to it, what are the molecular events occurring in the follicle to regulate the bursts of osteoclastogenesis? Consider the rat mandibular first molar as the model: An early event is the recruitment of the mononuclear cells to the dental follicle. Gene expression and immunostaining studies show that CSF-1 is maximally expressed in the rat follicle at day 3 post-natally, followed by a precipitous drop in subsequent days (Wise *et al.*, 1995). Monocyte chemotactic protein-1 (MCP-1) also shows a similar expression pattern (Que and Wise, 1997). Both CSF-1 and MCP-1 are chemokines for monocytes (Rollins *et al.*, 1988; Wang *et al.*, 1988; Yu and Graves, 1995), and, in subsequent *in vitro* studies, we demonstrated that both CSF-1 and MCP-1 are secreted by the dental follicle cells and are chemotactic for monocytes (Que and Wise, 1997). Gene microarray studies have confirmed this enhanced expression of CSF-1 and MCP-1 and have detected an increased expression of one other chemokine, endothelial monocyte-activating polypeptide 2 (EMAP-II), in the follicle at days 3 and 9 (Liu and Wise, 2007). Current studies are under way to determine if EMAP-II is also secreted by the follicle cells and is chemotactic for mononuclear cells. It should also be noted that once osteoclast precursors reach the dental follicle, the precursors themselves might elicit paracrine chemotactic signals, such as the chemokine CCL9 (*e.g.*, see Yang *et al.*, 2006).

The correlation between maximal expression of these chemokines in the rat dental follicle and the maximal number of mononuclear cells at day 3 is striking. Although correlation is not always causal, such a correlation in another species strongly suggests that these chemokines are central to the recruitment of mononuclear pre-osteoclasts to the follicle. In the mouse, the time of maximal mononuclear cell numbers in the follicle is at day 5, and that is the time that both CSF-1 and MCP-1 genes are maximally expressed in the follicle of the mouse (Wise *et al.*, 1999).

Enhancement of CSF and MCP-1 gene expression in the follicle at this early time post-natally, the day 3 rat and the day 5 mouse, may arise from the expression of other molecules in the stellate reticulum, the epithelium adjacent to the dental follicle. For example, molecules such

as transforming growth factor-beta 1 (TGF- β_1) and interleukin-1 α (IL-1 α) are expressed maximally early in the rat dental follicle (Wise *et al.*, 2002). In turn, TGF- β_1 and IL-1 α enhance MCP-1 expression in the dental follicle (Que and Wise, 1998), enhance synthesis and secretion of MCP-1 by the follicle cells (Wise *et al.*, 1999), and, in the case of IL-1 α , enhance the chemotactic ability of the dental follicle cells (Wise *et al.*, 1999). IL-1 α enhances the transcription of the CSF-1 gene in the dental follicle cells *in vitro* (Wise and Lin, 1994), and injection of IL-1 α enhances CSF-1 expression *in vivo* in the follicle (Wise, 1998).

It is of interest to note that, almost 20 years ago, Cahill *et al.* (1988) proposed that a “clock” existed in the enamel organ that regulated the cellular events of eruption. The stellate reticulum is a major component of the enamel organ adjacent to the dental follicle, and one could speculate that it is the secretion of such molecules as IL-1 α and/or TGF- β_1 with their subsequent effect on the dental follicle that initiates eruption. However, in null mice devoid of the IL-1 receptor gene, the teeth do erupt, although there is a slight delay in eruption (Huang and Wise, 2000). Thus, eruption can occur without the IL-1 α signal.

Another molecule localized to the stellate reticulum is parathyroid-hormone-related protein (PTHrP), and no tooth eruption occurs in the absence of its expression in the stellate reticulum (Philbrick *et al.*, 1998). However, we have recently shown that PTHrP is maximally expressed in the stellate reticulum at a later date (days 7-9), and thus may not initiate the onset of eruption (Yao *et al.*, 2007).

Continuing with the major burst of osteoclastogenesis, after the osteoclast precursors have been recruited to the dental follicle, how does the dental follicle initiate and regulate osteoclast formation? The first clue came from studies showing that the osteoprotegerin gene was constitutively expressed in the dental follicle of either the rat or mouse (Wise *et al.*, 2000). Most striking, however, was the fact that the osteoprotegerin was down-regulated in the dental follicle at day 3 in the rat and at day 5 in the mouse (Wise *et al.*, 2000). *In vitro*, either CSF-1 or parathyroid-related protein (PTHrP) could decrease osteoprotegerin gene expression in the follicle cells in both a time-dependent and concentration-dependent fashion (Wise *et al.*, 2000).

The decrease in osteoprotegerin expression in the rat dental follicle would allow maximal osteoclastogenesis to occur at day 3, as is indeed seen. The maximal expression and secretion of CSF-1 at day 3 are likely the reason for the down-regulation of osteoprotegerin at day 3. To determine if CSF down-regulated osteoprotegerin *in vivo*, we compared osteopetrotic toothless (*tl*) rats, deficient in CSF-1, with their normal littermates at given ages (days 1, 3, 5, 7, 9, 11), to determine if the osteoprotegerin expression was greater in the *tl* rat. If CSF-1 does inhibit osteoprotegerin gene expression, the absence of functional CSF-1 would result in a greater expression of osteoprotegerin in the *tl* rats than in the normal littermates. This indeed was the finding, and demonstrated that CSF-1 down-regulated CSF-1 *in vivo* (Wise *et al.*, 2005). In conjunction with this, *in vitro* studies also showed that transfecting the dental follicle cells with a short interfering RNA specific for CSF-1 mRNA resulted in an up-regulation of osteoprotegerin expression (Wise *et al.*, 2005).

Although the drop in osteoprotegerin in the dental follicle is viewed as the key regulatory event allowing osteoclastogenesis to occur at day 3, some RANKL, as well as the CSF-1 present, is needed to drive osteoclast formation. Laser capture microdissection and RT-PCR studies showed that RANKL was expressed in the dental follicle *in vivo* (Yao *et al.*, 2004), and a subsequent real-time RT-PCR study showed that RANKL was expressed in the dental follicle post-natally, with maximum expression at day 9 (Liu *et al.*, 2005). The fact that RANKL is present at day 3, but not maximally expressed until later, reinforces the concept that the decrease in osteoprotegerin expression effected by CSF-1 at day 3 is critical for enabling the major burst

of osteoclastogenesis to occur; *i.e.*, only by decreasing osteoprotegerin would a favorable ratio of RANKL/osteoprotegerin for osteoclastogenesis be established.

Recent gene microarray studies suggest that another inhibitor of osteoclastogenesis, SFRP-1, is present in the dental follicle and is down-regulated at days 3 and 9 post-natally (Liu and Wise, 2007). *In vitro* osteoclastogenesis assays show that maximal inhibition of osteoclast formation occurs when both anti-osteoprotegerin and anti-SFRP are present, suggesting that osteoprotegerin and SFRP may use different mechanisms to inhibit osteoclastogenesis (Liu and Wise, 2007). Regardless, the fact that both osteoprotegerin and SFRP gene expression are down-regulated at day 3 again emphasizes that the reduction of osteoclast inhibitors is critical for the major burst of osteoclastogenesis to occur.

In summary, Fig. 3 qualitatively depicts the levels of gene expression, in the dental follicle, that initiates and regulates the major burst of osteoclastogenesis at day 3 for the first mandibular molar of the rat. Maximal levels of MCP-1 and CSF-1 at day 3 promote the recruitment of osteoclast precursors to the dental follicle, where CSF-1 and RANKL can then stimulate osteoclastogenesis. Most importantly, the high level of CSF-1 reduces osteoprotegerin expression, such that the inhibition to osteoclastogenesis is reduced. The level of another inhibitor, SFRP1, is also reduced, but it is not yet known what molecule inhibits SFRP1.

The regulation of the minor burst of osteoclastogenesis at day 10 by the dental follicle requires some new molecules. The gene expression of CSF-1 is greatly reduced from its highs of day 3 (Fig. 3) (Wise *et al.*, 1995), and, although not shown in the Fig., MCP-1 expression is also reduced (Que and Wise, 1997). Replacement of some of the functions of CSF-1 appears to be accomplished by vascular endothelial growth factor (VEGF). VEGF and its 2 major isoforms (VEGF 120 and 164) are maximally expressed in the dental follicle at days 9-11 (Wise and Yao, 2003a). Another gene, tumor necrosis factor alpha (TNF- α), is also maximally expressed at day 9 in the dental follicle (Wise and Yao, 2003b), and, *in vitro*, TNF- α up-regulates VEGF gene expression (Wise and Yao, 2003b).

In the major burst of osteoclastogenesis, CSF-1 appears to play a role in recruiting osteoclast precursors, depressing osteoprotegerin gene expression in the dental follicle, stimulating proliferation of osteoclast precursors, and stimulating RANK production in the osteoclast precursors. VEGF cannot do all of this. It has been reported that it can recruit osteoclasts to the site of injection of VEGF in osteopetrotic mice (Niida *et al.*, 1999; Kaku *et al.*, 2000). Whether it can recruit osteoclast precursors to the dental follicle is unknown. It can up-regulate the expression of RANK in endothelial cells (Min *et al.*, 2003), and we recently have shown that it can up-regulate RANK expression in osteoclast precursors (Yao *et al.*, 2006). This is likely one of its major roles for the minor burst of osteoclastogenesis.

VEGF appears not to down-regulate osteoprotegerin gene expression, because the constitutive level of osteoprotegerin expression during the minor burst of osteoclastogenesis does not decrease (see Fig. 3). *In vitro*, osteoclastogenesis assays also show that, in the presence of RANKL, purified spleen mononuclear cells are not induced to form osteoclasts to any significant degree when VEGF is added (Yao *et al.*, 2006). In contrast, osteoclast formation is induced if CSF-1 is added to RANKL, and even greater numbers are seen if both CSF-1 and VEGF are present (Yao *et al.*, 2006). Finally, VEGF cannot stimulate proliferation of the osteoclast precursors *in vitro* (Yao *et al.*, 2006).

In essence, VEGF likely substitutes fully for CSF-1 to induce RANK formation. The small amount of CSF-1 present at day 10 likely is enough to interact with VEGF to help promote the minor burst of osteoclastogenesis.

The RANKL gene expression in the dental follicle is maximally up-regulated on days 9-11 (Liu *et al.*, 2005). Although it is not known what molecule(s) may up-regulate RANKL expression in the dental follicle at this time, TNF- α is a possible candidate, because it does up-regulate RANKL gene expression in the dental follicle cells *in vitro* (Liu *et al.*, 2005), and because it is also maximally expressed at day 9 (Wise and Yao, 2003b), a time that correlates with the maximal RANKL expression.

That RANKL production in the dental follicle affects alveolar bone resorption is supported by studies in which mice null for RANKL were transfected with a CD4 enhancer to drive expression of RANKL in B- and T-lymphocytes, but not in the dental follicle cells. In such rescued mice, bone resorption was seen in the long bones, but no alveolar bone resorption or tooth eruption was seen (Odgren *et al.*, 2003). Thus, it is the production of RANKL in the dental follicle itself that appears to be needed for the promotion of osteoclastogenesis and resorption of alveolar bone.

It is likely that the maximal expression of RANKL at days 9-11 creates a favorable ratio for the minor burst of osteoclastogenesis, despite the high levels of osteoprotegerin present (Fig. 3). With the VEGF and a small amount of CSF-1 also present, *in vitro* studies show that osteoclastogenesis can occur (Yao *et al.*, 2006).

Although not shown in Fig. 3, another putative eruption molecule, PTHrP, may play some role in the minor burst of osteoclastogenesis. Laser capture microdissection and RT-PCR studies have shown that PTHrP is maximally expressed in the stellate reticulum at days 7-9, and *in vitro* PTHrP can enhance VEGF gene expression in the dental follicle cells (Yao *et al.*, 2007). Others have also suggested that PTHrP can up-regulate RANKL expression in dental follicle cells (Nakchbandi *et al.*, 2000), but we have not been able to show this in our dental follicle cell lines. Regardless, PTHrP may have other functions in tooth eruption as well. For example, in PTHrP-gene knock-out mice or in tooth germs treated with an antisense oligonucleotide against PTHrP, there are few osteoclasts around the tooth germ, and bone spicules invade the tooth germ (Liu *et al.*, 2000; Kitahara *et al.*, 2002). Thus, the authors suggest that PTHrP may protect the tooth germs from bone invasion and subsequent ankylosis. PTHrP may also affect bone formation (osteogenesis), as will be discussed later.

Although this review has focused on the chronology of the expression of tooth eruption genes in the regulation of osteoclastogenesis, the regional localization of the genes within the dental follicle may be of equal importance. One only has to examine the ultrastructure of the alveolar bony crypt in which the unerupted tooth resides to see that two very different activities are occurring at opposite poles of the crypt—*i.e.*, bone resorption in the coronal one-half and bone formation in the basal (apical) one-half (Fig. 4). Bone architecture reflects the physiological state of the bone (Boyde and Hobdell, 1969), and scanning electron microscope studies of the bony crypt of the 3rd and 4th premolars of the dog showed that the coronal region of the bony crypt appeared scalloped (indicating bone resorption), whereas the basal region was trabecular (indicating bone formation) (Marks and Cahill, 1986). Similar findings were observed for the alveolar bony crypt of the first mandibular molar of the rat (Wise *et al.*, 2007). Thus, it was postulated that the coronal one-half of the dental follicle would regulate bone resorption, and the basal one-half of the dental follicle would regulate bone formation, a hypothesis supported by the finding that removal of only the coronal one-half of the follicle would prevent bone resorption and tooth eruption (Marks and Cahill, 1987).

To test this hypothesis of regional areas of the dental follicle regulating different functions, we conducted studies using laser capture microdissection (LCM) and real-time RT-PCR in which the coronal one-half of the follicle was excised and compared with the excised basal one-half. Looking at the gene expression of RANKL as a marker for osteoclastogenesis and bone

resorption, beginning at day 3, we found that the coronal one-half had a higher expression of RANKL than did the corresponding basal one-half for a given day (Wise and Yao, 2006). Conversely, when we examined the gene expression of bone morphogenetic protein-2 (BMP-2) as a marker for bone formation, BMP-2 expression was greater in the basal one-half than in the coronal (Wise and Yao, 2006). Thus, it appears that, in addition to tooth eruption requiring a precise chronology of gene expression, specific times at which various eruption molecules are either up- or down-regulated in the dental follicle, eruption also requires a difference in regional expression of the genes within the dental follicle.

Finally, in view of the fact that the dental follicle differentiates into the periodontal ligament, are any of the eruption genes of the dental follicle that regulate osteoclastogenesis expressed in the periodontal ligament? Numerous reports have indicated that key osteoclastogenic molecules—such as RANKL (Kanzaki *et al.*, 2001; Hasegawa *et al.*, 2002; Fukushima *et al.*, 2003), osteoprotegerin (Sakata *et al.*, 1999; Kanzaki *et al.*, 2001; Wada *et al.*, 2001; Hasegawa *et al.*, 2002), and VEGF (Oyama *et al.*, 2000)—are expressed in the periodontal ligament. The osteoprotegerin is secreted *in vitro* by the periodontal ligament fibroblasts and can inhibit osteoclast formation (Wada *et al.*, 2001). In essence, it appears that the constitutive synthesis of osteoprotegerin by the periodontal ligament would serve to prevent osteoclastogenesis of the alveolar bone, such that the periodontal ligament attachment would remain intact. Only during a period of tooth eruption would the osteoprotegerin expression need to be inhibited such that bone resorption could occur. In disease states such as periodontitis, however, RANKL levels are up-regulated and osteoprotegerin levels down-regulated (Crotti *et al.*, 2003; Liu *et al.*, 2003; Mogi *et al.*, 2004), such that the alveolar bone is resorbed. In essence, periodontitis mimics, to some extent, the osteoclastogenic events of tooth eruption.

In Orthodontic Tooth Movement

Osteoclastogenesis in orthodontic tooth movement is initiated by two related changes brought about by the application of force: tissue damage, with the subsequent production of inflammatory processes in the periodontal ligament; and deformation of the alveolar process. Osteoclasts and committed osteoclast progenitor cells, identified by the synthesis of tartrate-resistant ATPase and H(+)-ATPase immunohisto-chemistry, appear at sites of compression within days after forces are applied. Osteoclast induction, represented by mononuclear pre-osteoclasts, first occurs in vascular and marrow spaces of the alveolar crest, followed by increases in the periodontal ligament space (Yokoya *et al.*, 1997; Rody *et al.*, 2001). Their numbers correlate with finite element method (FEM) predictions of strains in the periodontal ligament and alveolar bone, with compression sites showing more than tension sites (Kawarizadeh *et al.*, 2004). Increases in proinflammatory cytokines (IL-1, 6, 8, and TNF α) also correlate well with this distribution (Alhashimi *et al.*, 2001; Bletsa *et al.*, 2006; Lee *et al.*, 2007), suggesting that cytokines are important initiators of osteoclastogenesis in tooth movement. Experiments have also demonstrated that these cytokines interact synergistically with bradykinin and thrombin in prostaglandin biosynthesis, thereby mediating inflammatory bone resorption (Marklund *et al.*, 1994; Ransjo *et al.*, 1998). There is also evidence that local administration of rhVEGF markedly enhances the number of osteoclasts at pressure sites during orthodontic tooth movement in osteopetrotic (op/op) mice (Kaku *et al.*, 2001), and that treatment with anti-VEGF antibody reduces osteoclast numbers and the amount of tooth movement (Kohno *et al.*, 2005). Analysis of these data suggests that the VEGF-CSF-1 mechanism, previously described in osteoclastogenesis associated with tooth eruption, may also be important in orthodontic tooth movement.

Changes in RANK, RANKL and osteoprotegerin have been demonstrated in the tooth-supporting tissues during orthodontic tooth movement (Oshiro *et al.*, 2002), with evidence of RANKL stimulation and osteoprotegerin inhibition of osteoclastogenesis (Kanzaki *et al.*,

2001). Compressive force up-regulates RANKL through a PGE₂ pathway, supporting osteoclastogenesis (Kanzaki *et al.*, 2002), while local osteoprotegerin gene transfer to the tooth-supporting tissues inhibits RANKL-mediated osteoclastogenesis and tooth movement (Kanzaki *et al.*, 2004). Increases in RANKL and the decreases in osteoprotegerin have also been demonstrated in cases of severe orthodontic root resorption, suggesting that this mechanism may be important in this negative sequelum of orthodontic treatment (Yamaguchi *et al.*, 2006).

Clearance of osteoclasts from compression sites occurs between 5 and 7 days following appliance activation in the rat (King *et al.*, 1991b). This is initiated in part by osteoclast apoptosis, followed by secondary necrosis (Noxon *et al.*, 2001). Physical forces act through specific receptor-like molecules—such as integrins, focal adhesion proteins, and the cytoskeleton—to activate certain protein kinase pathways (p38 MAPK and JNK/SAPK), which in turn amplify the signal and activate caspases, promoting osteoclast apoptosis. The cell phenotype and the character of the physical stimuli determine which pathways are activated and, consequently, allow for variability in response to a specific stimulus in different cell types (Hsieh and Nguyen, 2005). In addition to osteoclasts, osteocytes have been shown to undergo apoptosis at orthodontic compression sites (Hamaya *et al.*, 2002), but the details of how these two mechanisms may differ remain unclear. The latter is related to disuse (Bakker *et al.*, 2004), suggesting that the unloading of the principal fibers of the periodontal ligament at these sites may be important.

MOLECULAR REGULATION OF OSTEOGENESIS

In Tooth Eruption

As described earlier, the architecture of the alveolar bony crypt displays different regions of bone activity, as seen by SEM. In the sockets of the 3rd and 4th mandibular premolars of the dog, 3 distinct regions exist: (1) the coronal (superior) one-half, consisting of scalloped bone (resorption occurring); (2) the basal (apical) one-half, consisting of trabecular bone (formation occurring); and (3) a narrow smooth area between the two halves that is an inactive region (Marks and Cahill, 1986; Marks *et al.*, 1994). A similar SEM morphology is seen for the socket of the first mandibular molar of the rat (Fig. 3), except that the smooth area of bone is not always as well-defined (Wise and Yao, 2006; Wise *et al.*, 2007).

The manner in which the dental follicle might regulate this disparate bone activity at opposite poles of the bony crypt was first suggested by Marks and Cahill (1986), who postulated that the coronal region of the dental follicle might regulate alveolar bone resorption, whereas the basal region of the dental follicle would regulate bone formation (osteogenesis). They followed this up with a study in which they surgically removed either the coronal one-half or the basal one-half of the dental follicle of the dog premolar and examined the effect on eruption. Removal of the coronal one-half of the dental follicle resulted in no alveolar bone resorption and no tooth eruption, and removal of the basal one-half resulted in no bone growth and no tooth eruption (Marks and Cahill, 1987). These studies dramatically demonstrated that both bone resorption and bone formation are required for eruption. Equally important, it appeared that the coronal portion of the dental follicle regulated bone resorption, whereas the basal portion of the dental follicle regulated osteogenesis.

The molecular regulation of osteogenesis by the dental follicle has recently begun to be elucidated, thanks in large part to laser capture microdissection, which allows one to excise specific regions of the dental follicle of different ages and then examine gene expression of these excised regions using real-time RT-PCR. Thus, a molecule that promotes osteoblast formation and osteogenesis, BMP-2 (Wang *et al.*, 1990; Chen *et al.*, 1998; Gori *et al.*, 1999) was examined. BMP-2 was expressed in the follicle (Wise *et al.*, 2004), and comparison of the

coronal vs. basal halves for a given age showed that, beginning at day 3 post-natally, BMP-2 was expressed more in the basal one-half than in the corresponding coronal one-half for a given day, other than for day 7 (Wise and Yao, 2006).

The correlation between BMP-2 expression in the dental follicle and bone growth at the base of the socket further supports the view that the basal one-half of the dental follicle regulates osteogenesis, and that BMP-2 is a critical molecule needed for osteogenesis. In a recent SEM study of the alveolar bony crypt of the rat 1st mandibular molar, trabecular bone (osteogenesis) was seen at the base of the crypt beginning at day 3, and extensive trabecular bone was seen at the base at day 9 (Wise *et al.*, 2007). At both of these times, the level of BMP-2 gene expression in the basal half of the dental follicle exceeded that in its coronal counterpart (Wise and Yao, 2006). However, at day 7, the base of the crypt was relatively smooth, and it is on this day in which there was no significant difference between the coronal and basal halves in terms of BMP-2 gene expression (Wise and Yao, 2006; Wise *et al.*, 2007). Thus, a strong correlation exists between BMP-2 expression in the basal one-half of the dental follicle and the presence of trabecular bone (osteogenesis) in the basal portion of the socket.

The presence and/or role of other potential osteo-inductive molecules in the dental follicle has not yet been examined. The expression of a critical transcription factor for osteoblast differentiation, core-binding factor $\alpha 1$ (Cbfa1) or Runx2, has been observed in the dental follicles of mice (D'Souza *et al.*, 1999; Bronckers *et al.*, 2001). Although mice null for Cbfa1 die at birth, heterozygotes, Cbfa1 (+/-), sometimes display a delay or failure of eruption (see review by Wise *et al.*, 2002). Although Cbfa1 may be expressed in the dental follicle, the eruption delays in heterozygotes may be due to osteoblast defects. Regardless, the importance of osteogenesis in eruption is again emphasized.

Finally, the significance of osteogenesis in eruption, and an indirect molecular regulation of it, comes from studies of membrane-type 1 matrix metalloproteinase (MT1-MMP). Mice deficient in MT1-MMP display delayed tooth eruption (Beertsen *et al.*, 2002; Bartlett *et al.*, 2003). In both studies, alveolar bone resorption occurs, but alveolar bone growth does not. MT1-MMP degrades collagens I, II, and III, as well as other extracellular matrix molecules (d'Ortho *et al.*, 1997), which, in turn, affects the remodeling of bone. In particular, Beertsen *et al.* (2002) found that periodontal ligament fibroblasts in the MT1-MMP-deficient mice show a large accumulation of phagosomes containing collagen fibrils. Thus, in the periodontal ligament (a dental follicle derivative), an appropriate remodeling of its connective tissue and the bone interface likely is needed for alveolar bone formation to occur (Beertsen *et al.*, 2002).

In Orthodontic Tooth Movement

Tensile strains determine osteogenic activity, and the nature of the applied loads determines osteoblast recruitment (Fig. 2B). Static loads do not seem to play an important role in skeletal osteogenesis. Instead, osteogenesis is driven by bouts of loading above a threshold, and the most important characteristics of those loads are their strain rates, amplitudes, and durations (Forwood and Turner, 1995). At first, osteogenesis related to tooth movement seems unusual, because many orthodontic appliances are designed to deliver static, or slowly dissipating, loads. However, it is important to realize that the dentition is exposed to multiple changing loading bouts during mastication, swallowing, and speech, suggesting that the loads applied to the dentition are rarely static.

Much like tooth eruption, osteogenesis associated with orthodontics is mediated by various osteoinductive molecules. In general, most of these molecules are regulated by tensile strains and act by stimulating osteoblast progenitor cell proliferation in the periodontal ligament, subsequent bone formation, and the inhibition of bone resorption. Molecules that have been

linked in this way to orthodontic tooth movement include TGF β (Brady *et al.*, 1998), various BMPs (Mitsui *et al.*, 2006), bone sialoprotein (BSP) (Domon *et al.*, 2001), and epidermal growth factor (EGF) (Guajardo *et al.*, 2000; Gao *et al.*, 2002). Although the precise mechanisms at work in orthodontic osteogenesis have not been extensively examined, reasonable inferences can be made from the extensive body of literature on bone mechanotransduction that will be discussed in the next section of this review.

UNDERLYING BIOLOGICAL AND BIOMECHANICAL MECHANISMS

Motive Force of Tooth Eruption

In discussions of tooth eruption, the use of the word “force” must be used carefully. Eruption is both a physiological and developmental event, and, as such, these events are the products of biological processes that may include growth, differential growth, apoptosis, cell migration, etc. In some instances, force may be a secondary event resulting from a biological process, but it is imperative that the underlying biological mechanisms be recognized as the required elements in eruption.

What are the biological mechanisms that result in the tooth emerging from the bony crypt in which it is encased, such that it ultimately reaches its occlusal plane? For the intra-osseous phase of eruption, in which the tooth moves out of its bony crypt to pierce the gingiva, the two processes discussed extensively in this review—osteoclastogenesis and osteogenesis—are required. Without bone resorption as a result of osteoclastogenesis, no eruption pathway forms, and the tooth cannot escape its bony crypt, as seen in osteopetrotic rodents or experimentally where alveolar bone resorption is inhibited (see review by Wise *et al.*, 2002). Without alveolar bone formation, teeth do not erupt (Marks and Cahill, 1987; Beertsen *et al.*, 2002; Bartlett *et al.*, 2003).

Alveolar bone formation at the base of the tooth socket during tooth eruption has long been known to occur, as demonstrated elegantly in studies in which dog premolars were temporarily impacted (Cahill, 1969b). After release, there was extensive bone growth at the base of the socket as the teeth erupted. Later studies with microradiography and fluorescence microscopy also demonstrated alveolar bone growth at the base of the crypt (Pilipili *et al.*, 1995).

A detailed SEM study of the alveolar bony crypt of the first mandibular molar of the rat confirmed that extensive bone growth occurs at the base of the crypt during the intra-osseous phase of eruption (Wise *et al.*, 2007). Beginning at day 3, trabecular bone was seen at the base of the crypt, and by day 9 the crypt began to be reduced in depth as a result of this bone formation. By day 14, the bone almost filled the length of the crypt, to form the interradicular septum (Fig. 5). This extensive growth of the interradicular septum has also been observed in human molars (Sicher, 1942). Thus, in essence, this deposition of new bone only at the base of the crypt during the intraosseous phase of eruption leaves no place for the tooth to go but coronally, toward the eruption pathway (Fig. 5).

Although one could argue that the bone growth is not causal, the various studies cited earlier, showing that teeth do not erupt without alveolar bone growth, indicate that it is causal. Moreover, other biological processes previously suggested as a biological mechanism of eruption during the intra-osseous phase likely are not valid. These include the following:

1. root elongation as a force of eruption—Rootless teeth can erupt (Gowgiel, 1961; Marks and Cahill, 1984);
2. periodontal ligament as a force of eruption—In the rat molar, the dental follicle does not become organized into a periodontal ligament and attach to the cementum and alveolar bone until the intra-osseous phase of eruption is complete (Wise *et al.*,

2007). The same is true for dog premolars (Cahill and Marks, 1982). In addition, an inert metal replica can erupt, and there is no periodontal ligament attachment to it (Marks and Cahill, 1984). Transection of fibers in the dental follicle prior to the onset of eruption does not affect eruption rates or movement (Cahill and Marks, 1980); and

3. vascular pressure—Regional changes in vascular pressure have long been proposed as a force of eruption, but the evidence for this is both inconclusive and contradictory (see review by Cahill *et al.*, 1988; Marks and Schroeder, 1996). In more recent studies, Cheek *et al.* (2002) showed, in human 2nd premolars, that injection of a vasodilator above the root apex caused a transient increase (30 min) in the rate of eruption, whereas injection of a vasoconstrictor caused a decrease or intrusion. Similarly, in rat mandibular incisors, systemic infusion of angiotensin II increased mean arterial pressure, decreased regional blood flow, and decreased the eruption rate (Shimada *et al.*, 2004). Conversely, a positive correlation was found between the eruption rate and increased regional blood flow.

Unfortunately, it is difficult to reconcile these studies with the fact that an inert object minus pulp and roots can erupt—*i.e.*, there are no vessels in the pulp to affect eruption (Marks and Cahill, 1984). Another concern is that pharmacologic agents are often used, and the eruption changes they induce are only transient. Any type of pressure could briefly move a tooth, and one has to question if this reflects the long-term physiological process of eruption. Contradicting the pressure studies, as well, are experiments which have shown that hypotensive drugs have no effect on eruption (Main and Adams, 1966).

Mechanotransduction

Unlike tooth eruption, the motive forces at play in orthodontic tooth movement are primarily mechanical. The various appliances used in treatment and the actions of the oro-facial musculature generate them. Although there are few studies directly addressing the mechanotransduction mechanism in orthodontics, we can learn much about the putative mechanisms involved by considering the research on mechanotransduction in other systems. For more thorough discussions of this topic, the reader is referred to several recent reviews (Moss, 1997a,b; Bloomfield, 2001; Hughes-Fulford, 2004; Klein-Nulend *et al.*, 2005).

There are four essential interrelated steps in the transduction of mechanical signals by tissues: sensing the mechanical signal by the cells, transduction of this mechanical signal into one that is biochemical, transmission of the biochemical signal to the effector cells, and the effector cell response.

Osteocytes have several characteristics that make them the most likely candidates for being the mechanosensing element in bone. They are located throughout bone tissue and have cellular processes that are shaped for the easy detection of substrate deformations; osteocyte cell processes are bathed in a pericanalicular fluid that is in a confined space and therefore susceptible to slight changes in flow brought about by mechanical perturbations; and osteocytic processes are connected to each other and to osteoblasts *via* low-resistance gap junctions that facilitate the transmission of signals throughout the tissue (Burger and Klein-Nulend, 1999).

Fluid flow in bone canaliculi is highly site-specific and relates to applied loads (Knothe Tate *et al.*, 2000). Local changes in pericanalicular permeability have been demonstrated to have implications for osteocyte viability and intercellular communication in bone. Loads increase the pericellular fluid flow of probes up to 70 kDa (Tami *et al.*, 2003), and oscillating fluid flow above that obtained by routine physical activities increases the number of cells responding in the lacunar-canalicular system by enhanced calcium ion mobilization and expression of osteopontin, suggesting that fluid flow may be an important signal in bone cell mechanosensing (You *et al.*, 2000). Two further lines of evidence support this conclusion: Oscillating fluid flow

inhibits the expression of RANK by bone cells (Kurokouchi *et al.*, 2001); and bone cells possess primary cilia that project from their cell membranes, deflect during fluid flow, and are required for osteogenic and bone-resorptive responses to dynamic fluid flow (Malone *et al.*, 2007).

Although both tissue deformation and fluid shear occur in loaded bone, these signals may excite different pathways. For instance, pulsating fluid flow increases both nitric oxide and PGE₂ levels, but cyclic substrate strain stimulates only the release of nitric oxide, having no effect on PGE₂. Furthermore, substrate strains enhance bone matrix collagen synthesis, while fluid shear causes a reduction in collagen synthesis (Mullender *et al.*, 2004). Furthermore, *in vitro* results indicate that short-term changes in PGE₂ in response to pulsatile fluid flow are not associated with long-term changes in osteogenesis (Nauman *et al.*, 2001).

Some also argue that osteocytes within bone tissue can control the recruitment of osteoclasts and osteoblasts by sending strain-related signals to trabecular surfaces through the osteocytic canalicular network (Ruimerman *et al.*, 2005). The expression of connexin 43, a gap junction protein, is elevated after orthodontic force application, suggesting that signaling *via* low-resistance gap junctions may be important in the coordination of remodeling events during orthodontic tooth movement (Su *et al.*, 1997).

The mechanosensing apparatus of bone becomes less sensitive to repeated strain applications (Hayashi *et al.*, 2004). Recently, experimental protocols that insert “rest” periods to reduce the effects of desensitization have demonstrated significant benefits by increasing anabolic responses to mechanical loading (Turner and Robling, 2005). These approaches also have been tried in experimental orthodontic tooth movement studies (Konoo *et al.*, 2001; Hayashi *et al.*, 2004; Nakao *et al.*, 2007) and suggest that the inclusion of rest periods in orthodontic treatment protocols may also have the important benefit of reducing tissue damage without sacrificing tooth movement.

The transduction of mechanical into biochemical signals is accomplished *via* the integrin-actin cytoskeletal mechanism. Recently, considerable progress has been made on the mechanism by which mechanical strains in substrates are transduced into biochemical signals. Substrate distortion initiates a conformational change in integrin $\alpha_v\beta_3$, with the activation of phosphoinositol 3-kinase, followed by an increase in integrin binding to extracellular matrix proteins. Mechanical stretch stimulation of the Jun N-terminal Kinase (JNK) signaling pathway is dependent on this new integrin binding to extracellular matrix (Katsumi *et al.*, 2005).

Furthermore, mechanical responses by cells also depend closely on the dynamic changes in the structural architecture of the cytoskeleton. The latter consists of a set of highly interdependent substructures consisting of cortex, stress fibers, intermediate filaments, microfilaments, microtubules, and focal adhesions. The cytoskeleton softens and stiffens in response to applied stress, altering the mechanical properties of cells in complex ways (Chaudhuri *et al.*, 2007). Elimination of any of the cytoskeletal substructures results in a loss of the cell's ability to make these changes in intracellular consistency (Milan *et al.*, 2006). Focal adhesions are protein aggregates that connect cytoskeletal actin to extracellular matrix (Adachi *et al.*, 2003). These grow in size and change orientation and morphology as actin fiber force increases (Besser and Safran, 2006; Endlich and Endlich, 2006). We are now beginning to acquire new insights into how these physical changes in the cytoskeletal complex accomplish intracellular signaling. First, tension created in the cytoskeleton in response to loading can alter the shape of the membrane lipid bilayer, resulting in changes in ion channel behavior (Hamill and Martinac, 2001). Furthermore, G-protein-coupled receptors can alter their conformations in response to various mechanical stimulations, independent of ligand binding (Chachisvilis *et al.*, 2006).

Recently, a mechanotransduction role has been described for alpha-smooth-muscle actin (SMA), an actin isoform that contributes to cell-generated mechanical tension in certain muscle and non-muscle cell types (*e.g.*, myofibroblasts). This is based on its ability to link these mechanosensory elements physically, to enhance force-induced expression (Wang *et al.*, 2006). In this instance, cells utilize a feed-forward amplification loop involving focal adhesions, the binding of the p38 MAP kinase to SMA filaments, activation of the Rho signaling pathway, and binding of serum response factor to the CArG-B box of the SMA promoter in the genome.

Intercellular propagation of signals represents the third step in mechanotransduction. Several signaling pathways are emerging as potentially important in bone mechano transduction, including cations associated with membrane ion channels, nucleotides, second messengers, and mitogen-activated protein kinase (MAP-kinase). Currently, a unified understanding of how these various pathways interact in mechanotransduction remains to be clarified.

The opening of mechanosensitive cation channels in osteoblasts and the activation of protein tyrosine kinases, notably FAK, have gained attention as possible transduction pathways. The high expression in osteoblasts of the large-conductance K⁺ channels (BK) and their ability to open in response to membrane stretch make them prime candidates for a bone mechanoreceptor (Rezzonico *et al.*, 2003).

The Wnt surface receptor, low-density lipoprotein receptor-related protein 5 (LRP5), has been suggested as a key regulator of bone mass. Bone mineral density and strength in response to mechanical loading were recently found to be reduced in Lrp5-null (Lrp5^{-/-}) mice, despite normal osteoblast recruitment at mechanically strained surfaces. This has been linked to a defect in the ability of these osteoblasts to synthesize the bone matrix protein osteopontin after a mechanical stimulus, suggesting that this signaling pathway may be important for the osteogenic response to loading (Sawakami *et al.*, 2006).

Extracellular nucleotides, released in response to mechanical or inflammatory stimuli, signal through P2 receptors in osteoblasts. P2X7 receptors are ATP-gated cation channels that can induce formation of large membrane pores. Disruption of the P2X7 receptor leads to decreased periosteal bone formation and insensitivity of the skeleton to mechanical stimulation. A novel signaling axis has recently been described that links these receptors through phospholipases to the production of lysophosphatidic acid (LPA), activation of Rho-associated kinase, and osteogenesis during mechano-transduction (Panupinthu *et al.*, 2007).

Nitric oxide and prostaglandins also have been implicated in mechanotransduction pathways. NO, a short-lived free radical that inhibits resorption and promotes bone formation, is released within seconds in response to mechanical strain by both osteoblasts and osteocytes (Bakker *et al.*, 2001). Calvarial bone cells have also been shown to release prostaglandins in response to alterations in fluid flow (Ajubi *et al.*, 1999). Furthermore, load-induced osteogenesis can be blocked by the prostaglandin inhibitor, indomethacin (Forwood, 1996), and agonists of the prostaglandin receptors will increase new bone formation (Hagino *et al.*, 2005).

Mitogen-activated protein kinase (MAPK) signal transduction pathways also seem to be important in the mechanical response of bone. MAPKs are rapidly induced by stretching to enhance phosphorylation of c-Jun and c-Fos. This induction is accompanied by increased, phospho-c-Jun-containing AP-1-binding activity. Since AP-1 is known to be important in regulating genes activated at the onset of osteoblast differentiation, some have argued that an interplay of distinct MAPKs targeting AP-1 components may dictate the osteogenic response to mechanical stimulation (Peverali *et al.*, 2001). This idea is further supported by the demonstration that the inductive effects on AP-1 protein production can be partly or completely

abolished by specific inhibitors of p38 mitogen-activated protein kinase (MAPK), MAPK kinase (MEK), and Rho-associated protein kinase (RhoK).

Effector bone cells, following mechanical loading, synthesize numerous molecules related to bone remodeling. Recently, osteopontin (OPN), a major non-collagenous bone matrix glycoprotein, has been shown to have particular significance in the response to mechanical loading (Terai *et al.*, 1999). Because it carries an Arg-Gly-Asp (RGD) motif that promotes cell attachment through integrins and CD44, some have argued that OPN may promote bone resorption by enhancing the attachment of osteoclasts to the bone matrix (Reinholt *et al.*, 1990). Furthermore, OPN also contains a stretch of 10 aspartic acid residues that others suggest could be an important calcium-binding domain that may participate in the regulation of calcification (Denhardt and Guo, 1993).

OPN expression in alveolar bone increases in orthodontic tooth movement (Terai *et al.*, 1999), and there is a decreased level of bone resorption in OPN knock-out mice (Ishijima *et al.*, 2002). Furthermore, the injection of RGD peptide during orthodontics reduces the number of osteoclasts (Nomura and Takano-Yamamoto, 2000) and inhibits both tooth movement and dental drift (Dolce *et al.*, 2003). Recently, the critical role of OPN in the process of bone remodeling associated with tooth movement has gained further support by the observation that bone remodeling is suppressed in OPN knock-out mice. Moreover, the analysis of expression in the promoter transgenic mice indicated the presence of an *in vivo* mechanical stress response element in the 5.5-kb upstream region of the OPN gene (Fujihara *et al.*, 2006).

FUTURE STUDIES

Tooth Eruption

Given the importance of osteoclastogenesis and osteogenesis in eruption, future studies likely will focus on further elucidating the molecular regulation of these processes. Although much is known about the regulation of the key molecules required for osteoclastogenesis, molecular studies on the regulation of osteogenesis by the dental follicle have just begun. In that vein, it is imperative that various analyses be conducted to determine what osteo-inductive genes are present in the dental follicle. Following this, studies are needed to determine when these genes are expressed in relation to the osteogenic events of eruption.

An unexplored arena is the supraosseous phase of eruption for teeth of limited eruption. What are the biological mechanisms involved in this phase? What genes are expressed in the periodontal ligament at this time that may regulate eruption? Is the mechanism similar to what may occur in incisors (Berkovitz and Thomas, 1969; Moxham and Berkovitz, 1974)? These and numerous other questions make this a fertile area for future studies.

From a therapeutic viewpoint, understanding the cell and molecular requirements of eruption is the first step in understanding various tooth-eruption disorders, such as are seen with impacted third molars, primary failure of tooth eruption, Hutchinson-Gilford Progeria syndrome, and cleidocranial dysplasia. It is quite likely that these disorders arise from a specific defect(s) in gene expression, such that either osteoclastogenesis or osteogenesis (or both) is impaired. Thus, an ultimate therapeutic approach may well involve gene therapy, whereby a given gene(s) is delivered locally to the unerupted tooth.

Of equal interest therapeutically is the role of the periodontal ligament in periodontitis. As discussed earlier, the dental follicle gives rise to the periodontal ligament, and many of the osteoclastogenesis genes that are expressed in the dental follicle are expressed in the periodontal ligament. In periodontal disease, however, the ratio of osteoprotegerin to RANKL is altered to favor RANKL, and this may contribute to the alveolar bone resorption seen in

periodontitis (*e.g.*, see Crotti *et al.*, 2003). Thus, therapies to promote the osteoprotegerin/RANKL ratio in favor of osteoprotegerin—*i.e.*, the normal state in the periodontal ligament—may well reduce the alveolar bone resorption of the disease.

Orthodontic Tooth Movement

In addition to research to further our understanding of molecular mechanisms in osteoclastogenesis, osteogenesis, and mechanotransduction in orthodontic tooth movement, another exciting thrust for future research will involve the translation of new knowledge to the clinic. This will have two main focuses: diagnostics and therapeutics. Better knowledge of the molecular events in tooth movement will provide clinicians with important new tools for monitoring biological responses to treatment. This should result in more efficient treatment with less risk of negative sequelae. In addition to diagnostics, improved knowledge will provide new leverage for better interventions, including both biomechanical and pharmacological approaches.

Diagnostics

Today, there are no convenient ways to monitor orthodontic biomechanics clinically. In this review, we have mentioned some of the evidence suggesting that changes in strain characteristics do markedly alter cellular responses. Undoubtedly, more data on this and its role in tooth movement will be forthcoming. Therefore, it seems reasonable that improved methods for monitoring orthodontic forces in real time and improving the finite element models of the strains created in the tissues will be important.

The monitoring of selected biomarkers in gingival crevicular fluid during orthodontic treatment shows considerable diagnostic potential. However, this approach presents some significant challenges—for example, obtaining samples that are uncontaminated by bacterial components or gingival inflammatory products, availability of sufficiently reliable and precise micro-assays for μL volume samples, sampling variation based on location and the sequence of sampling, and the development of instrumentation for reading samples in a clinical setting. Biomarkers chosen to follow autocrine/paracrine and effector cell activities have been successfully assayed in gingival crevicular fluid during tooth movement. Increases in bone-resorptive cytokines, IL 2, 6, and 8 (Basaran *et al.*, 2006), and TNF α (Lowney *et al.*, 1995) have been reported. Recently, IL-1 β and IL-1 receptor antagonist ratios in gingival crevicular fluid have been able to predict the velocity of orthodontic tooth movement in a clinical setting (Iwasaki *et al.*, 2001). Furthermore, gingival crevicular fluid levels of RANKL and osteoprotegerin seem to reflect increased osteoclastic activity in the periodontal ligament, with higher levels of the former and lower for the latter at 24 hrs following appliance activation (Nishijima *et al.*, 2006).

Bone-remodeling enzymes have also been assayed in gingival crevicular fluid and may reflect temporal and spatial difference in these activities during orthodontic tooth movement. Changes in acid and alkaline phosphatase levels seem to suggest early bone-resorptive activity followed by later formation, reminiscent of a bone-remodeling cycle (Insoft *et al.*, 1996). Elevations in MMP 1, 2 (Ingman *et al.*, 2005; Cantarella *et al.*, 2006), 8 (Apajalahti *et al.*, 2003), and cathepsin B (Sugiyama *et al.*, 2003) have also been reported, suggesting that they may be useful for clinical assessment of extracellular matrix degradation. The presence in gingival crevicular fluid of the proteoglycan, heparan sulphate, supports the view that proteoglycans may also be useful biomarkers of resorptive processes in alveolar bone (Waddington *et al.*, 1994).

Therapeutics

There are numerous reports of the effects of the administration of various drugs, hormones, and other biologically active substances on orthodontic tooth movement. These can be categorized under two general headings: substances that enhance tooth movement, and those

that impede it. The former group could be useful adjuncts to the conventional orthodontic biomechanical signals to improve treatment time and efficiency, while the latter would be useful to preserve anchorage and stabilize the dentition following treatment.

The successful pharmacologic approaches to regulating orthodontic tooth movement have attacked the problem principally by controlling osteoclasts. Since alterations in osteoclast numbers and activities correlate well with tooth movement and root resorption, these substances generally have proven to be successful. However, one problem with focusing on the osteoclast has been that these substances usually have similar effects on odontoclasts. As a consequence, when they inhibit root resorption, they also inhibit tooth movement, and, conversely, they may have an increased risk of root resorption when they enhance tooth movement. The development of new bioactive substances with the ability to enhance tooth movement while also preventing root resorption would be important.

Although pro-inflammatory cytokines have yet to be used to enhance tooth movement, the administration of soluble receptors to IL-1 and TNF α has been shown to reduce osteoclast numbers and tooth movement in rats (Jager *et al.*, 2005). Metabolites of vitamin D have the ability to increase osteoclastic activity, and their local administration can also enhance orthodontic tooth movement in rats through that mechanism (Collins and Sinclair, 1988). Several drugs that inhibit prostaglandin pathways are effective at reducing osteoclastic activities in the paradental tissues, reducing tooth movement, or limiting root resorption. These include aspirin, acetaminophen, ibuprofen, indomethacin, and clodronate (Kehoe *et al.*, 1996; Liu *et al.*, 2006). Targeting the α v β 3 integrin receptor can reduce the ability of odontoclasts to attach to tooth surfaces, and thereby may be an effective means for reducing root resorption during tooth movement (Talic *et al.*, 2006). Likewise, osteoprotegerin can reduce osteoclastic activity, tooth movement, and root resorption (Penolazzi *et al.*, 2006). Chemically modified tetracyclines have the ability to inhibit MMPs without any antibacterial effects. These inhibit osteoclast numbers at compression sites, possibly by increasing apoptosis or reducing migration. They have been shown to reduce orthodontic tooth movement (Bildt *et al.*, 2006) and root resorption (Mavragani *et al.*, 2005). Bisphosphonates act on osteoclasts to inhibit their resorptive activity. These also inhibit orthodontic tooth movement (Igarashi *et al.*, 1994; Liu *et al.*, 2002), but come with an added risk for osteonecrosis of the jaws. Human relaxin acts by increasing fibrous connective tissue turnover.

CONCLUSIONS

Tooth eruption occurs as the result of a programmed and localized expression of molecules needed for alveolar bone resorption and formation, whereas orthodontic tooth movement focuses on the expression of these molecules for resorption and expression after induction by a mechanical force (see Table 1 for a listing of these molecules). Despite the different stimuli for gene expression (innate signaling vs. mechanical), commonalities exist in terms of the genes ultimately expressed and the end results achieved (see Table 2). Thus, it is instructive for researchers in both arenas, tooth eruption and orthodontic tooth movement, to follow the literature in both disciplines.

ACKNOWLEDGMENTS

This work was supported by NIH grant DE08911-16 to G.E.W. and by funds from the UW Moore Reidel Professorship to G.J.K. The authors thank Ms. Cindy Daigle for processing the manuscript. This review is dedicated to Sandy Marks, Jr., and Don Cahill for their pioneering research in tooth eruption, and to Alton Moore and Richard Riedel for their dedication to orthodontic teaching and research.

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Figure 1.

Comparison of compression and tension zones. **(A)** SEM of resorption cratering in a rat maxillary first molar root adjacent to a compression zone with 40 cN of orthodontic force for 5 days. **(B)** SEM of a rat maxillary first molar socket. Osteogenesis in response to 40 cN of orthodontic tension for 5 days can be seen as bony spicules extending into the socket from the top of the image.

Figure 2.

Morphology of unerupted tooth vs. erupted tooth. **(A)** Longitudinal section of a rat first mandibular molar at day 3 postnatally, showing the loose connective tissue sac, the dental follicle (DF), that surrounds the 1st molar (M1). Note that the unerupted tooth is encased in alveolar bone (AB), and that a prominent stellate reticulum is present. **(B)** Fluorescence micrograph image of a rat maxillary first molar and adjacent tissue with 40 cN of orthodontic force for 5 days. Root (R), periodontal ligament (P), and alveolar bone at compression (C) and tension sites (T). Tetracycline bone markers (orange and yellow) have been incorporated into the alveolar bone to demonstrate the bone turnover patterns. Note that the tension side is largely osteogenic, while the compression side is marked by cratering of both the bone and root, indicating resorption; however, some of the craters are also osteogenic, indicating that remodeling is taking place.

Figure 3.

Graph depicting the expression of genes in the dental follicle of the rat 1st mandibular molar at various days post-natally. At day 3, the major burst of osteoclastogenesis, osteoprotegerin is down-regulated such that a favorable RANK/osteoprotegerin ratio is established to promote osteoclastogenesis. At this time, CSF-1 is maximally expressed, both to down-regulate osteoprotegerin expression and to promote osteoclast precursor recruitment and osteoclastogenesis. At day 10, the minor burst of osteoclastogenesis, VEGF is maximally expressed to stimulate RANK expression on osteoclast precursors, as well as to interact with RANKL and CSF-1 to promote osteoclastogenesis. At this time, RANKL is up-regulated such that a favorable RANKL/osteoprotegerin ratio could exist to stimulate this minor burst of osteoclastogenesis.

Figure 4.

High-power SEM view of a portion of the wall of the alveolar bony crypt of a 1st mandibular rat molar at day 3 post-natally, extending from the coronal region (C) of the wall to the basal region (B). Although only a small area of the large coronal region is shown, note elongated depressions (D) that give a scalloped appearance to the bone in that region, whereas in the basal region the bone consists of many narrow trabeculae (T). Scalloped bone is undergoing resorption, whereas trabecular bone reflects bone formation. Between the two is an intermediate region (I) of bone that is relatively smooth. Such smooth bone is more inert, or neutral, reflecting neither growth nor resorption.

Figure 5.

Low-power SEM view of the alveolar bony crypt of the rat 1st mandibular molar at day 14, showing the extensive bone growth that has occurred at the base, especially in the region of the interradicular septum (IR). Four pits represent where the 4 roots reside—mesial (M), distal (D), mesiolabial (L), and mesiobuccal (B). A prominent interdental septum (ID) is present that separates the crypt of the first molar from the 2nd molar (2M). Note that all the newly formed bone, such as seen in the IR or ID, is trabecular.

Table 1

Listing of the Relative Importance of Regulatory Factors in Tooth Eruption and Tooth Movement

Mediators	Intra-osseous Tooth Eruption	Orthodontic Tooth Movement
CSF-1	+++*	++
RANKL	+++	+++
OPG	+++	+++
IL-1	++	+++
TGF- β 1	+	+++
PTHrP	++	-
TNF- α	++	+++
VEGF	++	++
BMP-2	+++	++
SFRP-1	++	-

* Based on numerous studies that include gene deletions, timing of gene up-/down-regulation, immunocytochemistry, osteopetrotic rodents, and *in vitro* assays, a subjective scale is presented of the importance of various mediators in intra-osseous tooth eruption and orthodontic tooth movement. Scale values are as follows: (-) not required or insufficient data; (+) slight effect; (++) moderate effect; (+++) greatest effect.

Table 2

Listing of How the Similar Requirements Needed for Tooth Eruption and Tooth Movement are Achieved

Common Requirements	Intra-osseous Tooth Eruption	Orthodontic Tooth Movement
Intervening bioactive soft tissue Initiating signal Histology/pathology Osteoclastogenesis Osteogenesis	Dental follicle Developmental program Bone modeling CSF-1, VEGF, RANK/RANKL/osteoprotegerin Developmental program	Periodontal ligament Biomechanical Injury/repair w/remodeling & modeling Pro-inflammatory cytokines, RANK/RANKL/osteoprotegerin Skeletal mechanotransduction