

# The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt

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**SUMMARY** The first experimental investigation of orthodontic tooth movement was published by Sandstedt in 1904–1905. After 100 years, there is a good understanding of the sequence of events at both tissue and cellular levels and now the current focus of research is at the molecular level. The techniques of reverse transcription–polymerase chain reaction and *in situ* hybridization to detect mRNAs of interest have revolutionized tooth movement studies and an expanding list of antibodies and enzyme-linked immunosorbent assays directed against human and animal proteins will facilitate their identification in tissue sections and/or culture supernatants. Nevertheless, although this technology has greatly simplified research for the clinical and laboratory investigator, message is not always translated into protein, and the presence of a protein does not necessarily mean it is biologically active.

*In vivo* and *in vitro* methods have been widely used in tooth movement studies. However, data from *in vitro* models, in which the mechanical stimulus can be carefully controlled (tension versus compression; intermittent versus continuous), should be correlated with *in vivo* data from animal models. The current evidence suggests that downstream from the initial mechanotransduction event at focal adhesions which link the extracellular matrix to the cytoskeleton, mechanically induced remodelling is mediated by a complex feedback mechanism involving the synthesis of cytokines such as interleukin-1 (IL-1), IL-6, and receptor activator of nuclear factor  $\kappa$  B ligand by cells of the osteoblast and/or fibroblast lineages. These in turn act in an autocrine/paracrine fashion to regulate the expression of transcription factors, cytokines, growth factors, enzymes, and structural molecules involved in the differentiation, proliferation, and function of mesenchymal and other cell types. Contrary to the impression gained from the literature, tooth movement is not confined to events within the periodontal ligament. Orthodontic tooth movement involves two interrelated processes: (1) deflection or bending of the alveolar bone and (2) remodelling of the periodontal tissues.

## Introduction

It is now 100 years since a three-part article on the theory of tooth movement was published (Sandstedt, 1904, 1905). It seems appropriate therefore to review the progress in our understanding of the tissue, cellular, and molecular mechanisms involved in orthodontic tooth movement since that time. The literature is now extensive (for previous reviews, see Reitan, 1951; Davidovitch, 1991; Sandy *et al.*, 1993), which creates difficulties when attempting to present a coherent narrative. To add some structure to the subject, it will be addressed largely chronologically and from the biological viewpoint, with a focus on how knowledge has evolved with the discovery of new molecules and the introduction of new experimental techniques. This approach shows that, in common with the rest of the biological sciences, the methodology of tooth movement research has become progressively more reductionist, making the subject less accessible to the clinician.

The significance of mechanical stimuli in the maintenance and structure of skeletal tissues has been recognized since the middle of the 19th century (von Meyer, 1867; Wölff, 1892), and is the traditional starting point for any discussion

of bone remodelling and tooth movement. The skeleton is continuously remodelled during life by osteoclasts resorbing old bone and osteoblasts subsequently forming new bone. Bone resorption and bone formation are therefore said to be coupled, a process of renewing the skeleton while maintaining its structural integrity (Frost, 1963). Remodelling is orchestrated by cells of the osteoblast lineage and involves a complex network of cell–cell and cell–matrix interactions involving systemic hormones, locally produced cytokines, growth factors, many of which are sequestered within the bone matrix, as well as the mechanical environment of the cells.

Orthodontic and orthopaedic theory and practice have much in common. Both involve the remodelling of bone and both require a thorough understanding of bone biology, particularly the relationship between mechanical stress and the various cell types in bone. However, tooth movement is a more complicated process requiring changes in the periodontal ligament (PDL) as well as the supporting alveolar bone, tissues with different cell populations and remodelling characteristics. During locomotion and other forms of physical activity, the skeleton is loaded intermittently.

Alveolar bone is also loaded intermittently during mastication, but is subject to a continuous deformation or strain during orthodontic tooth movement, and if the applied force is of sufficient magnitude, bending of the alveolus will occur. In the skeleton, the relationship between mechanical stress and bone resorption is poorly understood and the application of mechanically induced strain appears to be primarily an osteogenic stimulus (Hërt *et al.*, 1971; Lanyon and Baggott, 1976). Furthermore, the osteogenic response of bone to external loading appears to be due to a reactivation of quiescent bone-lining cells (Pead *et al.*, 1988; Chambers *et al.*, 1993), and not dependent on a preliminary phase of bone resorption (Chow *et al.*, 1998). Alveolar bone in contrast undergoes both resorption and deposition during orthodontic tooth movement, the extent of which is dependent upon the magnitude, direction, and duration of the applied force.

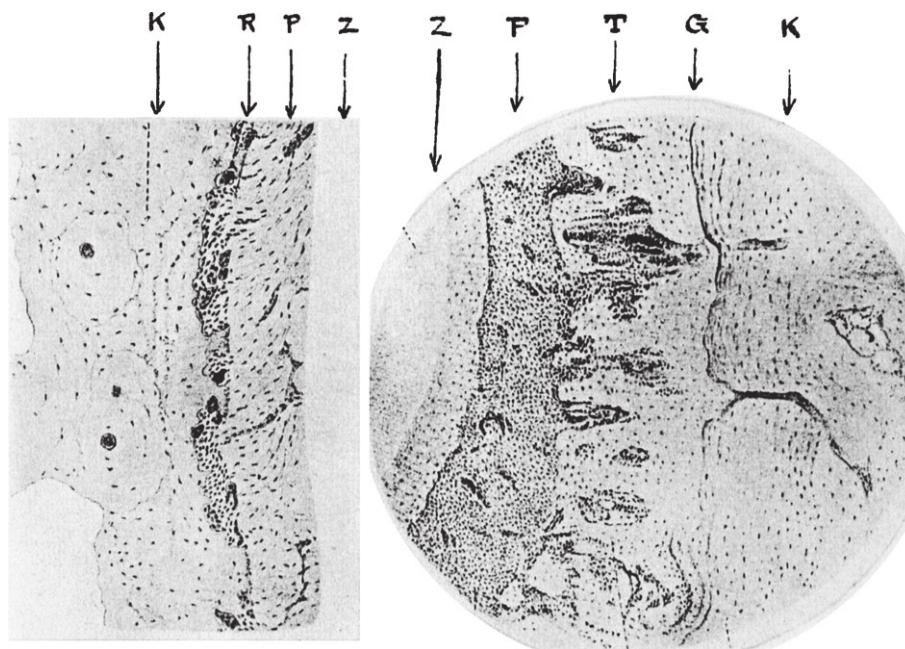
### Tissue reaction to orthodontic tooth movement

The idea that orthodontic tooth movement is dependent on the resorption and deposition of the bone of the socket dates back at least to 1839 (Harris, 1839). It was not until the turn of the 20th century, however, that the original histological investigation that forms the foundation of our present knowledge was carried out by Sandstedt (1904, 1905) on dogs and published shortly after his death (Persson, 2005).

### Laying the foundations: Sandstedt and Oppenheim

In Sandstedt's experimental model, a labial arch was bent to engage the six maxillary incisors of a dog and inserted into horizontal tubes attached to bands on the canines. The appliance was activated over a 3-week period by screws distal to the buccal tubes and during that time the crowns of the incisors were moved lingually by 3 mm. Sandstedt found that bone was deposited on the alveolar wall on the tension side of the tooth with both heavy and light forces, and that the newly formed bone spicules followed the orientation of the periodontal fibre bundles. On the pressure side, with light forces, alveolar bone was resorbed directly by numerous multinucleate osteoclasts in Howship's lacunae (Figure 1). With heavy forces, the periodontal tissues were compressed, leading to capillary thrombosis, cell death, and the production of localized cell-free areas of what he called hyalinization (owing to its glasslike appearance resembling hyaline cartilage in histological sections). At these sites, osteoclastic resorption of the adjacent alveolar wall did not take place directly, but was initiated by a process referred to by Sandstedt as 'undermining resorption' from the neighbouring marrow spaces.

Oppenheim (1911, 1930) published the results of experimental work carried out on the primary teeth of monkeys. His observations proved to be substantially different from those of Sandstedt as far as the response of the bone to compression was concerned. He found that



**Figure 1** Sandstedt's histological findings following the application of a light force to the upper incisors of a dog; tooth cut in cross-section. (Left) Pressure side. Z, root surface; P, compressed periodontal ligament; R, resorptive bone surface with numerous osteoclasts in Howship's lacunae; K, old alveolar bone with Haversian systems with no evidence of bone transformation as described by Oppenheim. (Right) Tension side. Z, root surface showing dentine and cementum; P, periodontal ligament; T, new bony trabeculae orientated along the principal fibres of the ligament; G, junction between new bone and old compact bones, K. (From Schwarz, 1932, *International Journal of Orthodontia, Oral Surgery and Radiography*.)

where a tooth had been tipped labially, the original bone disappeared completely from the labial surface and was replaced by new bone. He concluded that

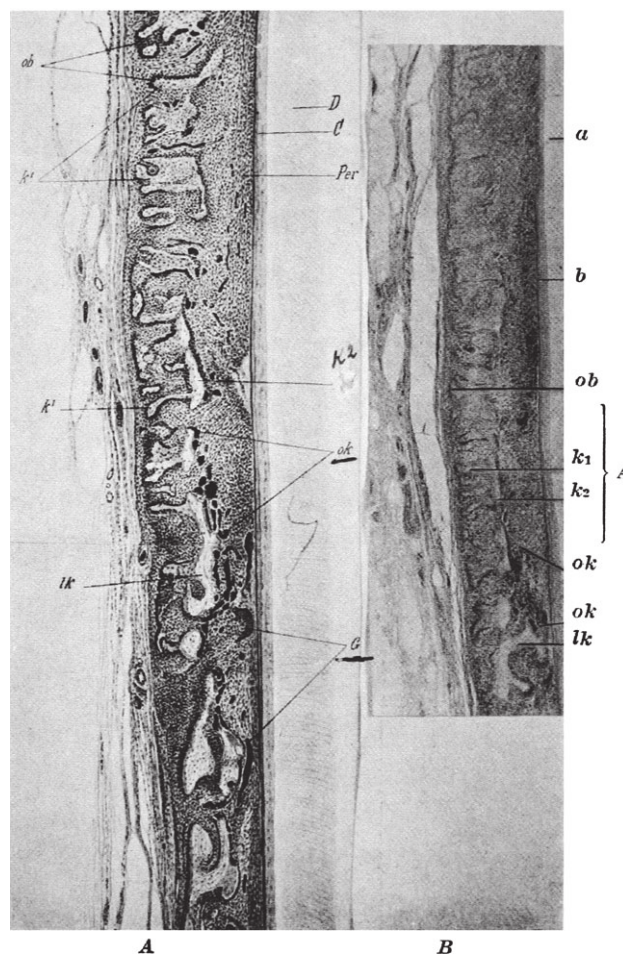
... bone tissue, be it compact or cancellated, reacts to pressure by a transformation of its entire architecture; this takes place by resorption of the bone present and deposition of new bone tissue; both processes occur simultaneously. Deposition finally preponderates over resorption.

Schwarz (1932) attempted to explain the difference between the findings of Sandstedt and Oppenheim by the fact that Oppenheim had killed his animals several days after the appliance had been last activated and did not therefore see the acute effect of the applied force, only a stage of regeneration after the force had been exhausted. It seems more likely that what Oppenheim was describing was the response of the periosteal and endosteal bone surfaces to the bending of the labial alveolar plate. The labial alveolar bone is particularly thin in his illustrations and would have been easily deformed in a monkey in the primary dentition (Figure 2). As discussed later, when bones are subjected to continuous mechanical deformation, concave surfaces are characterized by osteogenesis, and convex surfaces by bone resorption.

Whatever the explanation Oppenheim had lectured at the Angle School of Orthodontics and his research reported in the *American Orthodontist*, a journal published by the Angle Alumni Society (Oppenheim 1911, 1912). His theory of bone transformation also supported Angle's (1907) non-extraction philosophy and the widespread belief at the time that orthodontic appliances could 'grow bone'. Oppenheim's previous research on monkeys was reported again in 1930 together with the original photomicrographs and the findings of a new series of experiments carried out on dogs (Oppenheim, 1930). He also published articles on root resorption and repair in extracted human premolars following experimental tooth movement (Oppenheim, 1935, 1936). As a result, it was Oppenheim's work that appeared in mainstream English language orthodontic textbooks up until the 1950s and beyond. Sandstedt's early death and the lack of an English translation of his work did not help. He consequently became the forgotten man of tooth movement research until partially rescued from obscurity in the paper by Schwarz (1932).

#### *Reitan and the post-war period*

Following the work of Sandstedt and Oppenheim, reports by other investigators appeared in the literature (Gottlieb and Orban, 1931; Schwarz, 1932; Breitner, 1940), but it was not until the 1950s that tooth movement studies attracted wider attention. The leading histomorphological investigator of the period was the Norwegian orthodontist Kaare Reitan whose classic memoir was published in 1951 (Reitan, 1951).



**Figure 2** In 1930, Oppenheim republished his earlier research on tooth movement (which had been illustrated by drawings and criticized as such) together with the original photomicrographs. This is Figure 1 from the paper showing (A) the original drawing and (B) the photomicrograph of the bone changes following labial movement of a lower incisor for 40 days; tooth sectioned vertically. The text states ... 'It hardly leaves any doubt that while osteoclasts (*ok*) are absorbing bone on the inner wall, simultaneous formation of new bone is under way on the opposite side of the process.' At *ob*, near the crest of the alveolus, compact bone has disappeared and has been replaced by cancellous bone; the trabeculae of the latter have been arranged perpendicular to the long axis of the tooth; *k<sub>1</sub>*, new bone trabeculae lined with osteoblasts (*ob*). (From Oppenheim, 1930, *International Journal of Orthodontia, Oral Surgery and Radiography*.)

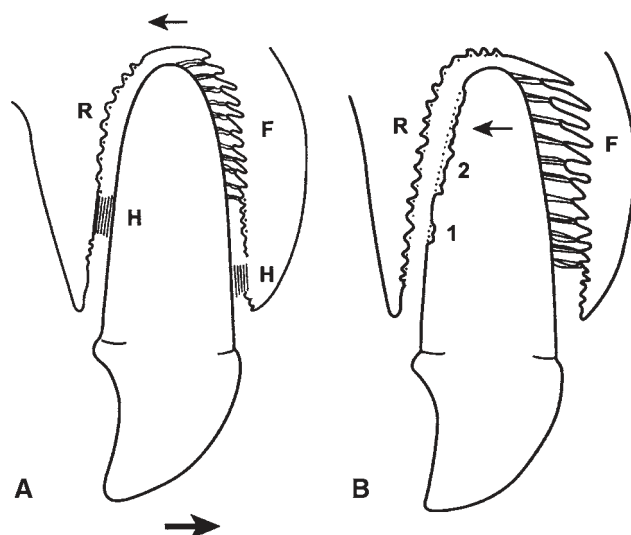
Reitan (1957, 1964) made extensive use of human material, particularly premolars that were destined for orthodontic extractions. His work highlighted the complexity of the tissue response to orthodontic treatment depending upon (1) the type (continuous versus intermittent) and magnitude of the force applied, (2) the mechanics involved (tipping versus bodily movement), and (3) the variation in tissue reaction between individual patients. He observed that during the initial stages of a tipping movement, cell-free or hyalinized areas were frequently created with a continuous force of 30 g. The time taken to remove such tissue by undermining resorption varied from 2 to 4 weeks and occasionally longer depending on the length of the root.

Intermittent forces of 70–100 g were also found to produce hyalinization. Cell-free areas were more common in tipping than in bodily movements presumably because, in the latter, the force was more evenly distributed along the root–bone interface (Figure 3). These experiments showed that even with an applied force as low as 30 g, some degree of hyalinization and root resorption appeared inevitable.

In a study of rotated teeth in young dogs, Reitan (1959) found that while the principal fibres of the PDL were rearranged or remodelled within 28 days, even after a retention period of 232 days, some of the free gingival fibres remained displaced and stretched. He concluded that rotational relapse was caused primarily by a contraction of the supra-alveolar gingival fibres (which contain elastic or oxytalin fibres) and advised the over-rotation and/or transection (percision) of these fibres during retention to ensure tooth stability.

#### The concept of differential force

Storey (1955a,b,c) who, in addition to analysing the histological changes produced by torsion springs applied to rodent and largomorph incisors, also carried out a series of experiments with canine retraction springs to determine what force levels should be used in clinical practice (Storey and Smith, 1952; Smith and Storey, 1952). In an investigation involving nine patients, they found that movement of the canine teeth into premolar extraction sites occurred rapidly when the value of the applied force was in the range of 150–250 g (5–9 ounces); however, below 150 g, the canines did



**Figure 3** (A) The application of a torquing force of 200 g to the second incisor of a dog resulted in tip and hyalinization (H) of the periodontal ligament (PDL) at sites of excessive compression. R, direct bone resorption; F, bone formation along the principal fibre bundles of the PDL. A torquing force of 200 g (B) was not accompanied by tip. R, resorption of bone along the surface of the alveolus and cementum and dentine of the opposing root surface (1, 2). Arrows indicate the direction of tooth movement. (Redrawn from Reitan, 1964.)

not move significantly. When the springs were activated to apply forces in the range of 400–600 g (14–21 ounces), the anchor teeth (molars and second premolars) moved forward, with the canines remaining relatively stationary.

These experiments gave rise to the differential force concept and the idea that there is an optimum range of force values that will produce the maximum rate of tooth movement. Subsequent research, however, found that the rate of canine retraction using the forces recommended by Storey and Smith (1952) and Smith and Storey (1952) was highly variable between individual patients (Hixon *et al.*, 1970; Boester and Johnston, 1974). This does not necessarily invalidate the concept as implied at the time, but indicates that the optimal force will be different for each patient. In any event, the magnitude of the applied force is just one of the many variables affecting the rate of tooth movement.

#### Autoradiographic investigations

One of the key technical developments during the 1960s as far as histomorphometry is concerned was the introduction of autoradiography. Tritium-labelled molecules, in particular, enabled, for the first time, changes in cell proliferation and metabolic activity to be measured with reasonable accuracy. Using a rat molar tooth movement model, Baumrind (1969) and Baumrind and Buck (1970) reported that cell proliferation (measured by  $^3\text{H}$ -thymidine incorporation) and metabolic activity ( $^3\text{H}$ -uridine incorporation) were increased, and protein synthesis ( $^3\text{H}$ -proline incorporation) was decreased, on both the 'tension' and 'pressure' sides of the PDL. This led them to question whether significant differences between the two sides actually existed.

Roberts and Jee (1974), Roberts *et al.* (1974), and Smith and Roberts (1980) used the same model to study the cellular kinetics of  $^3\text{H}$ -thymidine incorporation into PDL cells at tension sites. Smith and Roberts (1980) reported that over a time course of 20 hours, a continuous force produced a three-stage proliferative response. An interesting finding was a burst of mitotic activity within 2 hours, suggesting that the initial effect of mechanical strain was to allow  $\text{G}_2$ -blocked cells to enter the cell cycle and undergo mitosis, as well as  $\text{G}_1$ -blocked cells to synthesize DNA. The application of mechanical stress to the rat model also suggested that under strained conditions the cells of the PDL were primarily osteogenic (Roberts and Chase, 1981). This observation was supported by subsequent *in vitro* studies. PDL fibroblasts are functionally heterogeneous and contain a subpopulation of cells able to produce the osteoblast-related matrix proteins osteopontin, alkaline phosphatase (ALP), and bone sialoprotein (BSP; Lekic *et al.*, 2001; Murakami *et al.*, 2003).

#### Hyalinization and root resorption

The Scandinavian tradition of movement research was continued by Kvam (1972) and Rygh (1972a,b), with

particular emphasis on the cellular and tissue changes on the compression side. It had been realized by Sandstedt that hyalinization was related to changes in vasculature, and Gianelly (1969) also showed that resorption was dependent on the maintenance of patent vascular channels. Rygh (1972b) studied the ultrastructural changes in blood vessels in both human and rat material and found (1) packing of erythrocytes in dilated blood vessels within 30 minutes, (2) fragmentation of erythrocytes after 2–3 hours, and (3) disintegration of blood vessel walls and extravasation of their contents after 1–7 days. He also observed necrotic changes in PDL fibroblasts such as dilatation of the endoplasmic reticulum and mitochondrial swelling within 30 minutes, followed by rupture of the cell membrane and nuclear fragmentation after 2 hours; cellular and nuclear fragments remained within hyalinized zones for several days (Rygh, 1972a). There was no difference in the response of rat and human tissues apart from timing; changes observed after 2 days in humans were seen after 2 hours in rats.

Kvam (1972) and Rygh (1972a,b) showed that root resorption is a side-effect of the cellular activity associated with the removal of the necrotic hyalinized tissue. Scanning electron microscopy of premolar root surfaces following the application of a 50 g force to the crown in a lateral direction revealed resorption cavities extending into the dentine, but where the hyalinized tissue remained intact, the root surface was unaffected (Figure 4; Kvam, 1972). Most recently, tartrate-resistant acid phosphatase (TRAP) staining in a rat tooth movement model has highlighted the involvement of TRAP-positive macrophages and multinucleate giant cells in the removal of hyalinized tissue (Brudvik and Rygh, 1994), and clearly demonstrated that on reaching the adjacent root surface, TRAP-positive cells continue to remove the cementum and subjacent dentine-producing resorption lacunae (Figure 5).

### The pressure–tension hypothesis

The idea that pressure and tension sites are generated within the PDL is firmly embedded in the orthodontic subconscious and it continues to play a key role in organizing our ideas, as well as advancing our understanding of a complex biological process. However, there are two major conceptual problems associated with the hypothesis. First, does stretching of the principal fibre bundles generate tension and second, can differential pressures be developed within the tissues of the periodontium?

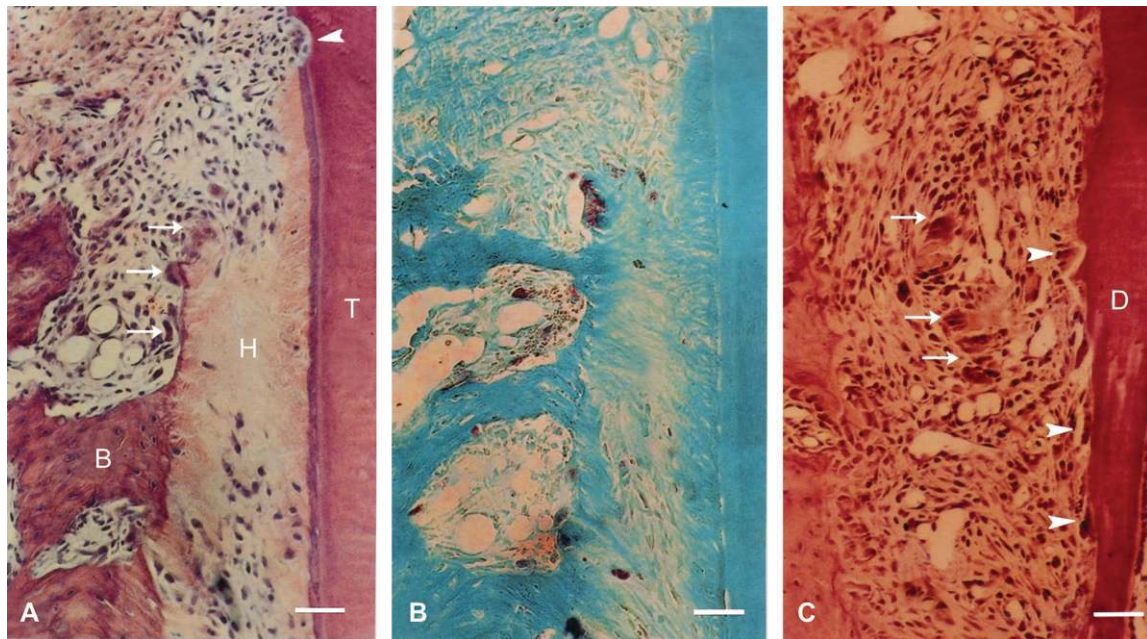
*Does stretching of the principal fibres of the PDL generate tension?*

A persistent dogma of the orthodontic literature is that the collagen fibres of the PDL are stretched during tooth movement. Tension is thereby generated in the fibres responsible for the cellular response, particularly the stimulation of osteogenesis at the cortical bone surface into which the fibres are inserted. This belief seems to have been partly due to the traditional textbook representation of a tooth suspended in its socket by the PDL, which overemphasized the role of collagen in tooth support, and also due to a lack of knowledge of the structure and function of the ligamental proteoglycans and other non-collagenous structural molecules that are not easily visualized by conventional histological processing.

Direct measurements of the experimental intrusion of teeth (Parfitt, 1960; Bien and Ayres, 1965; Picton, 1965) suggested that, when mechanically loaded, the periodontal tissues behave as a viscoelastic gel which flows when subjected to a steady force but ‘bounces’ when a load is briefly applied and then removed. The damping of the mechanical forces acting on a tooth was originally attributed to three distinct but interacting fluid systems: (1) the vascular system, (2) cells and periodontal fibres, and (3) the interstitial fluid continuum (Bien, 1966). While these fluid systems undoubtedly play a

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**Figure 4** Scanning electron micrographs of premolar root surfaces following experimental tooth movement in which a force of 50 g was applied in a lateral direction. Teeth were from patients aged 10–12 years who required extractions for orthodontic treatment. (A) Resorption cavity extending into the dentine after 76 days. Original magnification:  $\times 58$ . (B) Resorption defects after 35 days. The area covered by hyalinized tissue (c) was unaffected.  $\times 23$ . (Reprinted from Kvam, 1972, with permission from Blackwell Publishing.)



**Figure 5** Neighbouring sections from the compressed area of the mesio-lingual root of a rat maxillary first molar after tooth movement for 7 days. The hyalinized zone (H) between the alveolar bone (B) and root (T) reveals a fibrillar structure. Resorption of alveolar bone occurs from the marrow spaces (arrows). Note the resorption lacuna in the dentine at the periphery of the hyalinized zone (arrowhead). (A) Haematoxylin and eosin stain. (B) tartrate-resistant acid phosphatase (TRAP) stain highlighting TRAP-positive cells in the adjacent narrow spaces and at the margin of hyalinized tissue. (C) Compressed area after 10 days. Hyalinized tissue almost removed with resorption lacunae on both the bone and dentine surfaces. The multinucleate cells within the necrotic tissue (arrows) and lining the surface of the dentine (arrowheads) were shown in adjacent sections to be TRAP positive. Haematoxylin and eosin stain. Bars measure 50  $\mu\text{m}$ . (Reprinted from Brudvik and Rygh, 1994, with permission from Oxford University Press.)

part, the shock-absorbing function of the ligament is more likely to result from the ability of the hydrophilic proteoglycan molecules to form a strongly hydrated space-filling gel, whose displacement is limited by the collagen fibre network and lamina dura of the alveolar bone. Momentary pressures created by the sudden forces of mastication are therefore of different physiological significance to the prolonged pressures of orthodontic appliances, since biting forces in the neighbourhood of 1500  $\text{g}/\text{cm}^2$  do not crush the periodontal membrane and impact the tooth through bone (Bien, 1966).

To test the hypothesis that tension generated in the collagen fibres of the PDL provides the stimulus for osteogenesis, Heller and Nanda (1979) disrupted collagen metabolism and function in rats by the systemic administration of the lathyritic agent  $\beta$ -aminopropionitrile; this inhibits the intermolecular cross-linking of the polypeptide chains of the collagen molecule. They found that in lathyritic rats the histological response of the bone to orthodontic tooth movement appeared to be normal. This suggests that when a tooth is continuously loaded during orthodontic treatment, it is unlikely that the principal fibres of the PDL undergo significant tensile strain, or transfer forces directly to the alveolar bone via Sharpey's fibres.

#### *Can differential pressures be generated within the PDL?*

Baumrind (1969) proposed that since the PDL appears to behave as a continuous hydrostatic system, any force

delivered to it will, in accordance with Pascal's law, be transmitted equally to all regions of the ligament. But is the analogy appropriate? This depends on how one defines a fluid. To what extent can the supporting structures of a tooth (cells, collagen, proteoglycans, blood vessels, tissue fluids) be regarded as a fluid? And can the lamina dura of the tooth socket with its numerous vascular perforations be regarded as a closed vessel? Apart from minor adjustments following normal occlusal loading (and perhaps even then), the evidence from tooth movement experiments (localized vascular stasis, hyalinization, direct versus undermining bone resorption) would seem to overwhelmingly support the hypothesis that differential pressures can be generated within the periodontium.

#### **The role of bone bending in orthodontic tooth movement**

Baumrind (1969) also observed that the crown of the first molar was displaced, on average, 10 times more than the average reduction in PDL width on the pressure side, suggesting that bone deforms more readily than the PDL. Chapter 6 in Angle's Seventh Edition (Angle, 1907, p. 132) begins with the following paragraph:

When a force is exerted upon the teeth to be moved two principal changes take place in the alveolar process. First, a bending of the process; second, absorption of the

process in advance of the moving teeth and deposition of bone behind it. These changes vary greatly: according to the age of the patient, in different patients of the same age, in the direction of movement and also in the rapidity of movement.

The role of bone bending in orthodontic tooth movement was then ignored for the next 50 years, apparently even by Angle.

#### *Bone deflection studies*

Laboratory investigations that demonstrated unequivocally that bone deflection accompanied tooth displacement (Mühlemann, 1954; Mühlemann and Zander, 1954; Picton, 1965) were not undertaken with orthodontics in mind, but were physiological studies of tooth support. Experiments of Mühlemann and Zander (1954) with *Macaca mulatta* (rhesus) monkeys found that forces of 50–100 g were required to initiate labial or lingual displacement of a tooth from its physiological rest position and deformation of the alveolar bone started when forces greater than 100 g were applied to the teeth. Picton (1965) measured the distortion of alveolar bone with horizontal and axial forces in adult monkeys and noted that bone displacement started in response to forces less than 100 g and occurred in a linear manner up to 1 kg. Horizontal forces of more than 50 g caused the labial and lingual plates to be displaced in the same direction as the applied force; however, intrusive forces of the same magnitude caused dilatation of the socket, making it difficult to explain tooth support in terms of a tensional mechanism.

Although most bone deflection studies have been carried out on laboratory animals, experiments in humans have shown that the interseptal bone can also be bent. Measurements of interseptal bone deformation adjacent to tooth extraction sites were undertaken in two 12-year-old patients by Grimm (1972). Apart from variations in the rate and magnitude of tooth movement, bending of the alveolar crest was detectable with forces of less than 50 g and reached 35  $\mu\text{m}$  with a load of 1.5 kg. Somewhat surprisingly, he recorded a 5 to 30  $\mu\text{m}$  initial deflection of the crestal bone in the opposite direction to the tooth movement; this effect was transitory, however, and reversed to a slow positive trend after 1 minute.

#### *The cellular and molecular response to bone deflection*

Osteocytes are sensitive to mechanical deformation, and the idea that the cytoplasmic syncytium may detect changes in mechanical loading and therefore act as mechanoreceptors in bone has a long history (Pritchard, 1956). Experimental studies using both *in vitro* and *in vivo* models indicate that osteocytes are sensitive to stress applied to intact bone and may indeed function as mechanoreceptors. Load-related increases in glucose-6-phosphate dehydrogenase,

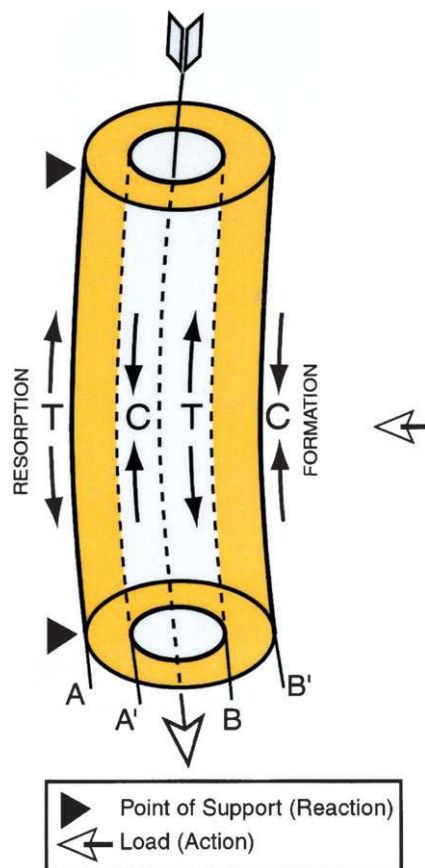
$^3\text{H}$ -uridine, c-fos, and insulin-like growth factor-I expression have been detected in osteocytes within 6 hours (Skerry *et al.*, 1989; El Haj *et al.*, 1990; Lean *et al.*, 1996), demonstrating that intermittent loading at physiological strain magnitude produces rapid changes in metabolic activity.

Recently, Pavlin *et al.* (2001), Pavlin and Gluhak-Heinrich (2001), and Gluhak-Heinrich *et al.* (2003) focussed on the temporal expression of osteoblast- and osteocyte-associated genes following the loading of alveolar bone in a mouse molar tooth movement model. They found the response of osteoblast-associated genes to deformation in cells at the PDL–bone interface to be 10- to 20-fold greater than the increase in their number, suggesting that differentiation and increased cell function were the initial responses to loading the bone. ALP and BSP gene expression were detected after 24 hours, followed by the concomitant stimulation of osteocalcin and collagen type I expression from 24 to 48 hours (Pavlin *et al.*, 2001). Most interesting, however, was the response of the osteocytes; the expression of dentine matrix protein-1 mRNA in the osteocytes of alveolar bone was increased twofold as early as 6 hours after loading, on both the formative and resorptive sides of the tooth (Gluhak-Heinrich *et al.*, 2003).

#### *Stress-generated electrical effects in bone*

When an external load is applied to a long bone, deformation occurs, thus producing changes in the surface curvature (Figure 6). The external surface of the left cortex elongates in tension, while the internal surface shortens in compression; opposite effects are produced on the internal and external surfaces of the right cortex (Epker and Frost, 1965). Increasing concavity has consistently been shown to be associated with electronegativity and bone formation. In contrast, increasing convexity is associated with electropositivity and bone resorption (Bassett and Becker, 1962).

Stress-generated electrical potentials have been recorded with surface electrodes in a dog mandible following the application of a mechanical force to the teeth (Gillooly *et al.*, 1968), and Zengo *et al.* (1973) proposed that electrical potentials are responsible for regulating osteogenesis and bone resorption in the course of orthodontic tooth movement (Figure 7). During the 1960s and 1970s, the regulation of bone remodelling by piezoelectrical effects enjoyed considerable support within the orthopaedic and orthodontic communities. However, the problems from a biological point of view are whether electrical phenomena are sufficiently discriminatory to be able to regulate the metabolic activity of cell types as diverse as osteoblasts and osteoclasts, which function in close proximity, and also the fact that piezoelectricity does not require the presence of living cells; dead bone displays the same effects, which appear to be generated by shearing forces acting on the collagen fibres of the bone matrix. The more that is learnt about cell–cell and cell–matrix interactions, the more it is

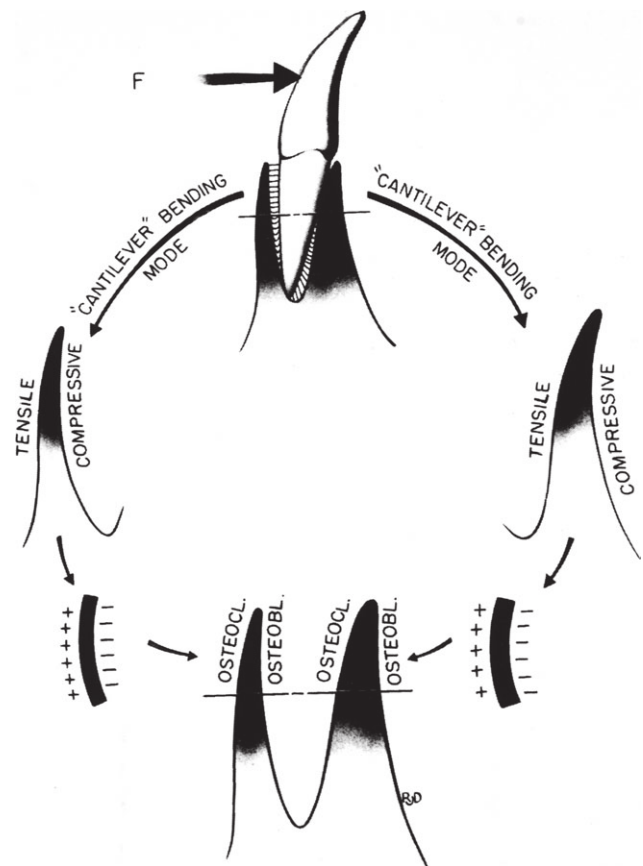


**Figure 6** Deformation of a long bone by a mechanical load from the right. The cortical bone surfaces A and B are curved convexly and the surfaces A' and B' curved concavely. The arrows show the direction of stress and strain; C, compression surface; T, tension surface. Bending causes surface bone resorption and formation, making the bone 'drift' to the right until the deforming load has disappeared. (Modified from Epker and Frost, 1965.)

likely that stress-generated electrical potentials represent a by-product of deformation, a physical phenomenon that provided a plausible explanation for bone remodelling before the discovery of growth factors, cytokines, and other locally produced biochemical mediators. Nevertheless, while piezoelectricity as an explanation for the regulation of bone remodelling may have been incorrect, Figure 7 highlights the manner in which the alveolar plates are deformed when a tooth is loaded horizontally, as well as the sites at which bone deposition and resorption take place.

### Surrogate models of tooth movement

It is clear from the preceding discussion that by the 1970s, numerous well-documented studies of the histology of tooth movement had been undertaken in animals. Animal experimentation had limitations, however, and if significant progress were to be made in understanding the cellular and molecular mechanisms involved, then different experimental

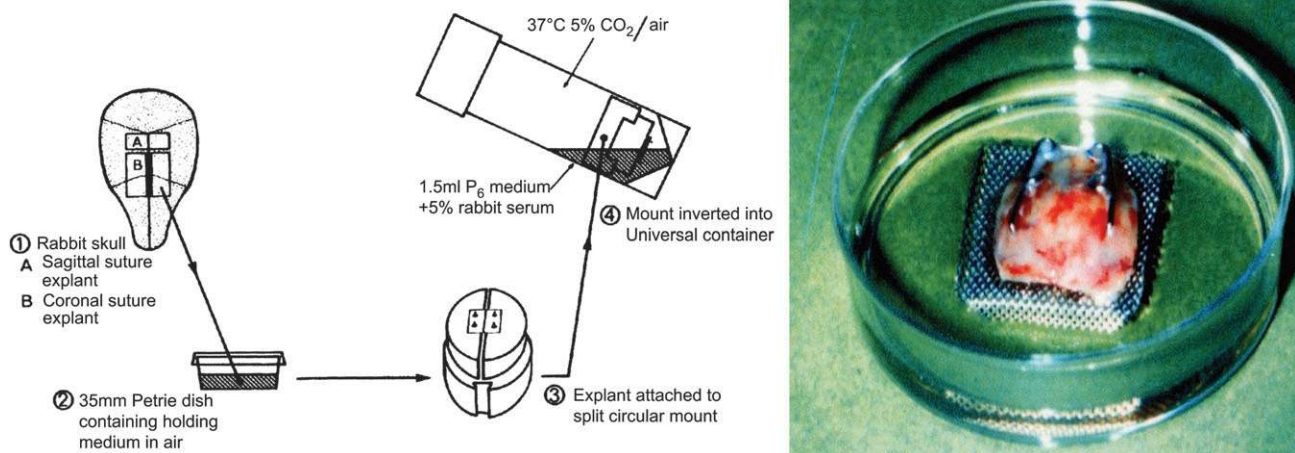


**Figure 7** Hypothetical model of the role of stress-induced bioelectric potentials in regulating alveolar bone remodelling during orthodontic tooth movement. The force, F, applied to the labial surface of the lower incisor displaces the tooth in its socket, deforming the alveolar bone convexly towards the root at the leading edge, and producing concavity towards the root at the trailing edge. Concave bone surfaces characterized by osteoclastic activity are electropositive or electrically neutral. (Reprinted from Zengo *et al.*, 1973, with permission from the American Association of Orthodontists.)

methods would be required. This provided the impetus for the development of *in vitro* cell and tissue culture systems designed to answer questions not readily accessible from animal studies conducted *in vivo*. Some of the experiments investigating cell signalling and mechanotransduction in which osteoblasts and PDL fibroblasts have been mechanically deformed in culture will be described later. This section focuses on the use of cranial sutures as a model of tooth movement.

Melcher and Turnbull (1972) and Yen and Melcher (1978) developed an organ culture system for mouse molar explants, but the technical difficulties of isolating and maintaining an explant composed of teeth and bone *in vitro* and applying force to the PDL were formidable. Meikle *et al.* (1979) developed organ culture systems in which mechanical force could be applied to rabbit cranial sutures under controlled conditions (Figure 8). The aim was to produce a simple experimental model that would mimic the forces to which the periodontal and other sutural articulations





**Figure 8** (Left) Experimental procedure for culturing fibrous joints from rabbit calvaria. Explants were cultured in 1.5 ml of a modified form of BGJ medium supplemented with 5 per cent heat-inactivated rabbit serum and ascorbate (150  $\mu\text{g}/\text{ml}$ ). Incubations were carried out at 37°C in a roller-tube culture apparatus rotating at 1 revolution per minute and continuously gassed (5 per cent  $\text{CO}_2$ , 95 per cent air, flow rate 50 ml per minute). A tensile stress was applied to the suture by a split circular mount and a coil spring. (Right) Sagittal suture explant cultured on a stainless steel grid in a 5-cm petri dish; a tensile stress was applied to the suture with a helical spring.

of the craniofacial skeleton are exposed during orthodontic treatment, and was based on the assumption that remodelling mechanisms are similar for all fibrous joints.

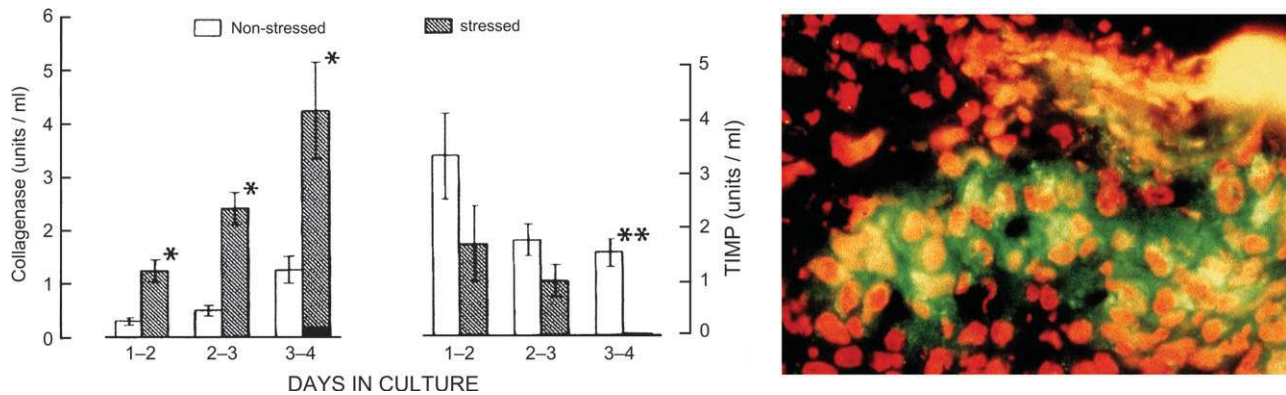
A particular advantage of the model was that both synthesis and degradation could be quantified contemporaneously using biochemical methods. Culturing explants in medium containing  $^3\text{H}$ -proline, for example, resulted in a two- to threefold increase in suture protein synthesis and a twofold increase in collagen synthesis that could be detected after 6 hours. There was also a significant increase in  $^3\text{H}$ -thymidine incorporation, and a threefold increase in the DNA content of the sutures after 48 hours, indicating a stimulation of cell proliferation and an increase in the cell population (Meikle *et al.*, 1979, 1984). While non-stressed sutures synthesized type I collagen, in stressed sutures, 20 per cent of the newly synthesized collagen was type III (Meikle *et al.*, 1982). Type III collagen is found in connective tissues with a high turnover and suggests that the biomechanical environment of a cell is an important determinant of the collagen type synthesized.

Surprisingly, cells under tensile strain did not preferentially synthesize structural proteins. Mechanical deformation also stimulated the synthesis of enzymes such as the matrix metalloproteinases (MMPs), collagenase, and stromelysin responsible for their specific hydrolysis, as well as down-regulating tissue inhibitor of metalloproteinase (TIMP) expression (Figure 9; Meikle *et al.*, 1980, 1984; Green *et al.*, 1990). These findings suggested that degradation of the extracellular matrix (ECM) was stimulated by fibroblasts under a continuous tensile strain. The functional significance of such a response is not clear, but it seems likely that increased matrix turnover is necessary to facilitate cell division and accommodate an increase in the cell population.

Although rendered largely redundant by the increasing availability of cDNA probes and antibodies to human, mouse, and rat proteins, recent *in vivo* studies in rats (Takahashi *et al.*, 2003) and *in vitro* studies with PDL fibroblasts (Bolcato-Bellemin *et al.*, 2000) have confirmed the above findings. Despite requiring a measure of lateral thinking, calvarial sutures continue to provide valuable information regarding the growth and remodelling of fibrous joints. In a mouse sagittal suture model for example, Ikegame *et al.* (2001) found that tensile mechanical stress increased bone morphogenetic protein-4 expression in preosteoblasts and fibroblasts, which in turn activated the expression of the osteoblast-specific transcription factor core-binding factor alpha-1, thereby inducing osteoblast differentiation.

### Mechanical strain activates multiple cell signalling pathways

One area in which *in vitro* methods continue to make a significant impact is in the expanding field of cell signalling. The earliest experiments showed that the immediate response of cells to mechanical strain was the generation of prostaglandins (PGs) and the second messengers 3',5'-cyclic adenosine monophosphate (cAMP; Harell *et al.*, 1977; Yeh and Rodan, 1984) and inositol phosphates (Sandy *et al.*, 1989). Changes in intracellular  $[\text{Ca}^{2+}]$  were later shown to occur via stretch-activated ion channels (Davidson *et al.*, 1990; McDonald *et al.*, 1996). Little was known at the time, however, about integrin receptors other than their role in cell adhesion. Activation of the cAMP and inositol phosphate signalling pathways is likely to lie downstream from the initial mechanoreception event at focal adhesions, where



**Figure 9** (Left) The effect of tensile mechanical stress on collagenase and tissue inhibitor of metalloproteinase (TIMP) production. Four pairs of coronal suture explants were mechanically deformed and culture supernatants collected daily over 4 days. Culture media were assayed for collagenase and TIMP activity using the standard fibril assay and show that as collagenase increases, there is a concomitant decrease in TIMP. Values are expressed as mean  $\pm$  standard error of the mean. (■) Denotes active enzyme. \* $P < 0.05$ , \*\* $P < 0.01$ . (Right) Immunolocalization of collagenase in a stressed coronal suture showing positive cells with diffuse fluorescence of the surrounding extracellular matrix indicative of the presence of active enzyme.

integrin receptors link the ECM to the cytoskeleton (Wang *et al.*, 1993; DeMali *et al.*, 2003).

#### The role of PGs

The first evidence that PGs might be involved in mechanical force transduction came from the cell culture studies of Hong *et al.* (1976), who observed that perturbing cell membranes by detaching cells mechanically from culture dishes caused an increase in PG synthesis. They suggested that mechanical distortion acts by changing the conformation of the cell membrane, thereby exposing membrane phospholipids to the action of phospholipases to release free arachidonic acid. These findings, plus research showing that changes in intracellular cyclic nucleotides followed mechanical deformation (Rodan *et al.*, 1975; Davidovitch and Shanfield, 1975), provided the impetus for an *in vitro* study by Harell *et al.* (1977). They deformed petri dishes with orthodontic screws cemented to the base on which osteoblast-like cells had been cultured and suggested the following sequence of events: (1) activation of phospholipase A<sub>2</sub> with the subsequent release of arachidonic acid resulted in a rapid (threefold) synthesis of PGE<sub>2</sub> at 5 minutes, (2) this in turn activated adenylate cyclase and a transient increase in intracellular cAMP which peaked at 15 minutes, and (3) an increase in intracellular [Ca<sup>2+</sup>] and a stimulation of DNA synthesis. The ability to mimic or inhibit these changes by exogenous PGE<sub>2</sub> or indomethacin [a cyclooxygenase-1 (COX-1) inhibitor] provided further evidence that the transduction of mechanical deformation into a biological response was mediated by PG production (Harell *et al.*, 1977; Somjen *et al.*, 1980). Other *in vitro* models have implicated PGs as mediators of mechanical stress in a number of cell types, including mechanically deformed osteoblasts (Yeh and Rodan, 1984), gingival fibroblasts (Ngan *et al.*, 1988), and PDL fibroblasts (Ngan *et al.*, 1990).

In an interesting practical application of the findings of Harell *et al.* (1977), Yamasaki *et al.* (1980) investigated the role of PGs in orthodontic tooth movement initially with the rat molar model. They found that indomethacin produced a dose-related inhibition in the appearance of osteoclasts and bone resorption that was limited to the first 12 hours of tooth movement. This explains why tooth movement is slower in patients who consume large quantities of non-steroidal anti-inflammatory drugs (NSAIDs). Injections of PGE<sub>1</sub> and PGE<sub>2</sub> into the gingival tissues near the upper first molar also stimulated osteoclast formation and bone resorption. This led to further work that showed local PG injections could increase the rate of tooth movement (Yamasaki *et al.*, 1982, 1984).

#### Leukotrienes

A rabbit model showed that the COX inhibitor flurbiprofen significantly reduced osteoclast numbers, but interestingly not the degree of tooth movement (Sandy and Harris, 1984). This paradoxical observation suggested that PGs alone did not account for the bone remodelling associated with tooth movement. Leukotrienes (LTs) are also metabolites of arachidonic acid (but produced via the lipoxygenase pathway) and could have accounted for this discrepancy since they are potent stimulators of bone resorption (Meghji *et al.*, 1988). It was subsequently shown that AA861, a selective inhibitor of the LT pathway, caused a significant inhibition of LTB<sub>4</sub> and a reduction in tooth movement in rats (Mohammed *et al.*, 1989).

#### The cAMP pathway

The second messenger system classically associated with mechanical force transduction is cAMP. The first evidence for the involvement of the cAMP pathway in mechanical signal transduction was provided independently by Rodan

*et al.* (1975) and Davidovitch and Shanfield (1975). Rodan *et al.* (1975) showed that a compressive force of 60 g/cm<sup>2</sup> applied to 16-day-old chick tibia *in vitro* inhibited the accumulation of cAMP in the epiphyses, as well as in cells isolated from the proliferative zone of the growth plate. The effect was mediated by an enhanced uptake of Ca<sup>2+</sup> which inhibited membrane-associated adenylyl cyclase activity (Bourret and Rodan, 1976).

Davidovitch and Shanfield (1975) sampled alveolar bone from compression and tension sites surrounding orthodontically tipped canines in cats. They found that cAMP levels initially decreased, followed by an increase after 1–2 days that remained elevated to the end of the experimental period of 28 days. They suggested that the initial decrease at the compression sites was due to necrosis of PDL cells, and at the tension sites to a rapid increase in the cell population; the elevation in cAMP observed 2 weeks after the initiation of treatment was probably a reflection of increased bone remodelling activity. Subsequently, Davidovitch *et al.* (1976), in a study on the cellular localization of cAMP in the same model, found an increase in the number of cAMP-positive cells in areas of the PDL where bone resorption or deposition subsequently occurred. Osteocytes in the adjacent alveolar bone, however, appeared to be relatively unaffected by the mechanical force.

#### The phosphoinositide pathway

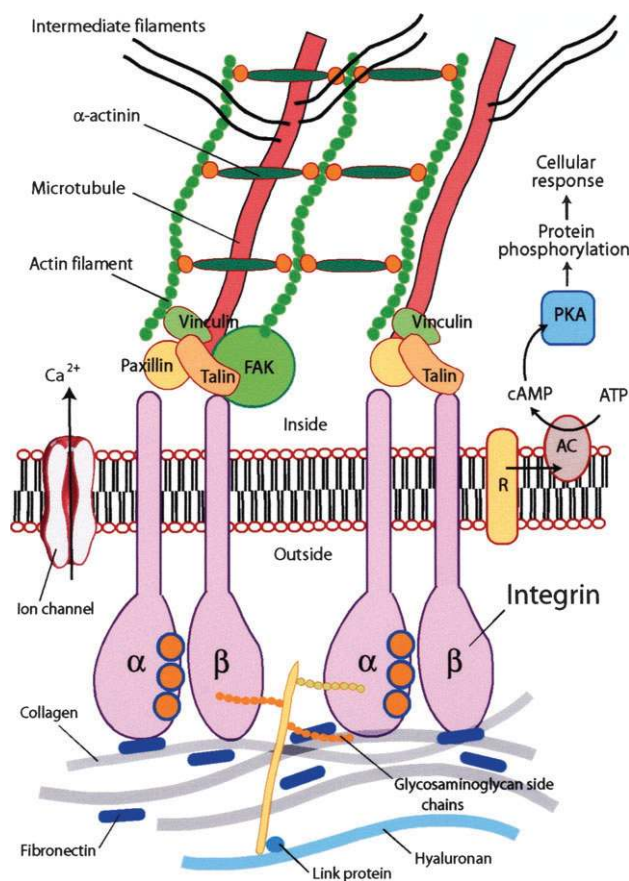
The phosphoinositide (PI) pathway had been investigated in the 1950s, but the importance of membrane phospholipids as cellular second messengers was not fully appreciated until the 1980s, when Streb *et al.* (1983) showed that products of inositol lipid breakdown caused a release of intracellular Ca<sup>2+</sup>. The PI pathway accounts for many of the changes seen in mechanically deformed tissues, including the elevation of intracellular [Ca<sup>2+</sup>] from the endoplasmic reticulum and increased DNA synthesis.

PGE<sub>2</sub> and parathyroid hormone (PTH) elevate inositol phosphates as well as cAMP in mouse calvarial osteoblast cultures; however, the effect of PTH on inositol phosphate mobilization was not direct, being mediated indirectly by PGs in an autocrine/paracrine fashion (Farndale *et al.*, 1988). Evidence that mechanical stress was also PG-mediated made it unlikely that only one pathway would be selectively mobilized, and subsequently, Sandy *et al.* (1989) found that both cAMP and inositol phosphates were elevated in mouse osteoblasts following intermittent mechanical strain. Activation of cAMP and inositol phosphates by PGE<sub>2</sub> has been shown to induce the expression of transcription factors such as Erg-1 (early growth response gene-1) and the proto-oncogenes c-jun and c-fos (referred to as immediate early genes). These activate or repress the transcription of late-acting genes required for bringing about changes in phenotype. Erg-1 is involved in cell

proliferation and has been shown to be elevated in rat osteoblasts by mechanical stretch within 15 minutes (Dolce *et al.*, 1996).

#### Signal transduction by integrins and focal adhesions

Cells in culture are not uniformly attached to their substrate but are ‘tack welded’ at specialized sites of cell attachment called focal adhesions. Focal adhesions are sites at which integrin receptors physically link actin-associated cytoskeletal proteins (talin, vinculin,  $\alpha$ -actinin, paxillin) with the ECM (Figure 10; Sastry and Burridge, 2000), as well as with adhesion molecules on the surface of adjacent cells. Integrins are therefore ideally sited to mediate the transmission of bidirectional forces across the cell membrane (Ingber, 1991). Integrins that are not bound to ligands in the ECM are generally distributed diffusely over the cell surface



**Figure 10** Diagram of integrin receptors and focal adhesions. Focal adhesions are sites where integrins link actin-associated cytoskeletal proteins (talin, vinculin,  $\alpha$ -actinin) and signalling molecules such as FAK and paxillin to the structural macromolecules of the extracellular matrix. Firm attachment at focal adhesions enables mechanical deformation of the cell to be recognized by (1) the cytoskeleton and intracellular signalling pathways and (2) mechanosensitive ion channels, phospholipids, and G-protein-coupled receptors in the plasma membrane. AC, adenylyl cyclase; FAK, focal adhesion kinase; PKA, protein kinase A; R, G-protein-coupled receptor.

and appear not to be linked to the actin cytoskeleton; association with the actin cytoskeleton is induced by binding to the ECM (Wang *et al.*, 1993). Integrins are composed of structurally distinct  $\alpha$  and  $\beta$  subunits that combine to generate heterodimeric receptors with unique binding properties; for example,  $\alpha_2\beta_1$  integrin binds to collagen and laminin and  $\alpha_v\beta_3$  to vitronectin. Integrins bind to adhesive and structural proteins in the ECM via the peptide sequence RGD (arginine–glycine–aspartate). Integrin-mediated adhesive interactions play key roles in cell migration, proliferation, and differentiation, and also regulate intracellular signal transduction pathways that control adhesion-induced (outside–in) changes in cell physiology (Wang *et al.*, 1993; Clarke and Brugge, 1995). Integrins thus function both as cell adhesion molecules and intracellular signalling receptors.

The numerous signalling pathways activated by integrins are beyond the scope of this discussion, but the low-molecular weight guanosine triphosphatases, Rab and Rho, as well as mitogen-activated protein (MAP) kinase subtypes that are components of integrin-mediated signalling have been shown to be altered in mechanically stretched PDL fibroblasts (Basdra *et al.*, 1995) and osteoblasts (Peverali *et al.*, 2001). MAP kinase phosphorylates and activates phospholipase  $A_2$ , leading to the hydrolysis of glycerophospholipids to yield arachidonic acid. Furthermore, integrin-dependent increases in intracellular  $[Ca^{2+}]$  appear to involve either inositol triphosphate-induced  $Ca^{2+}$  mobilization from the endoplasmic reticulum or the transport of extracellular  $Ca^{2+}$  through mechanosensitive ion channels (Clarke and Brugge, 1995).

Evidence is accumulating making it increasingly likely that the previously reported changes in cell signalling in response to mechanical deformation are downstream of events mediated by integrins at focal adhesions. In a study designed to determine whether cells sense mechanical signals through generalized membrane distortion or through integrins, Meyer *et al.* (2000) found that mechanical stress altered the cAMP signalling cascade and downstream gene transcription through signals generated by activated integrin receptors, and in a G-protein-dependent manner. In an earlier report (Wilson *et al.*, 1995), the proliferative response of cultured vascular smooth muscle cells to cyclic mechanical strain was found to be dependent on interactions between integrins and specific matrix proteins (collagen, fibronectin, vitronectin) since the response to strain was neutralized by antibodies to  $\beta_3$  and  $\alpha_v\beta_5$  integrins. Carvalho *et al.* (1996) have further shown that mechanical stimulation of bone cells results in a marked increase in  $\beta_1$  mRNA expression after 30 minutes ( $\alpha_v$  expression did not change). Firm attachment to the ECM at focal adhesions is evidently necessary to enable mechanical distortion to be recognized by (1) the cytoskeleton and intracellular signalling pathways and (2) mechanosensitive ion channels, phospholipids, and G-protein-coupled receptors in the cell membrane.

## Cytokines

One of the most significant advances in connective tissue biology during the 1980s was the demonstration that many cytokines, in addition to mediating the host immunological response to exogenous antigens, were also produced by connective tissue cells such as fibroblasts and osteoblasts and involved in normal physiological turnover and bone remodelling. Cytokines are low-molecular weight proteins (mw < 25 kDa) produced by cells that regulate or modify the action of other cells in an autocrine (acting on the cell of origin) or paracrine (acting on adjacent cells) manner. The definition includes the interleukins (ILs), tumour necrosis factors (TNFs), interferons, growth factors, and colony-stimulating factors. A major difficulty in understanding cytokine biology is the sheer number and complexity of these factors. Another problem is that several factors such as IL-1 and TNF exhibit overlapping biological activities (redundancy) and many have multiple biological effects (pleiotropy).

### *The role of cytokines in cell–cell signalling in bone*

Cells of the osteoblast lineage play a pivotal role in bone remodelling, a process that involves interactions between osteoblasts and osteoclasts, systemic hormones, cytokines, and growth factors. Systemic hormones and mechanical stimuli influence the process via their ability to control the synthesis and/or action of cytokines. Since bone remodelling occurs at discrete sites throughout the skeleton, osteoblast-derived cytokines are ideally placed to regulate or modify the action of other cell types in bone. The first cytokine shown to play a role in bone turnover was IL-1, which in addition to activating lymphocytes also proved to be a potent bone resorptive agent (Gowen *et al.*, 1983; Heath *et al.*, 1985). Not long afterwards, the TNFs were shown to stimulate bone resorption and inhibit bone formation *in vitro* (Bertolini *et al.*, 1986), and subsequent studies have implicated numerous others.

Experiments in which bone marrow had been cultured in the presence of bone resorptive agents such as PTH,  $1,25(OH)_2$  vitamin  $D_3$ , or IL-1 had shown that osteoclast formation and function was dependent upon the presence of osteoblasts/stromal cells. This suggested that cells of the osteoblast lineage produced soluble factors that were involved in osteoblast–osteoclast signalling. After many attempts to purify, characterize, and sequence such an osteoclast-activating factor using biochemical methods, a cell-surface protein that was found to be identical to receptor activator of nuclear factor  $\kappa$  B ligand (RANKL, a member of the membrane-associated TNF ligand family) was simultaneously discovered by two research teams (Lacey *et al.*, 1998; Yasuda *et al.*, 1998) using molecular techniques. RANKL and its receptor RANK expressed on osteoclasts and their precursor cells turned out to be the

molecular determinants of osteoclast formation and function (Figure 11).

Cell–cell signalling by RANKL is essential for the induction of osteoclast differentiation. PTH and other systemic hormones as well as cytokines, such as IL-1, TNF- $\alpha$ , and IL-6, stimulate bone resorption by their ability to up-regulate RANKL expression by osteoblasts/stromal cells. Another cytokine osteoprotegerin (OPG; Simonet *et al.*, 1997) also produced by osteoblasts/stromal cells acts as an inhibitor of osteoclast function by competing with RANKL for the membrane receptor RANK. Evidence is emerging which suggests that RANKL and OPG produced by PDL fibroblasts and osteoblasts play important roles in regulating connective tissue turnover and bone resorption during orthodontic tooth movement.

#### *Cytokines as mediators of mechanically induced bone remodelling*

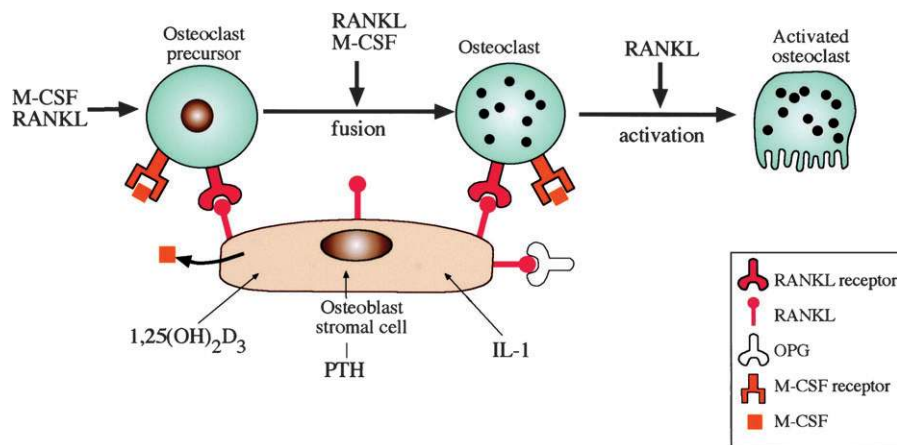
Early research into the effects of tensile strain on cellular metabolism showed, with few exceptions, that whatever was measured always seemed to increase. These observations suggested that the cellular response to mechanical stress was part of a generalized increase in metabolic activity, rather than a specific response to mechanical strain. Recently, however, Rubin *et al.* (2000, 2002) have shown that a tensile strain applied intermittently to mouse bone marrow stromal cells in culture decreased RANKL mRNA expression by approximately 60 per cent and suggested that strain-induced reductions in RANKL contributed to the maintenance of bone mass by physical activity. Additionally, a recent study of IL-10 and IL-12 expression (two cytokines

that inhibit osteoclast formation) has shown that within 2 hours of the application of intermittent tensile stress to mouse calvarial osteoblasts *in vitro*, the synthesis of IL-10 was down-regulated (IL-10 also suppresses osteoblast differentiation) and IL-12 synthesis up-regulated (García-López *et al.*, 2005). In addition to showing that the synthesis of some cytokines can be suppressed by mechanical stress, these data support the conclusion that the initial response of osteoblastic cells to tensile strain is to promote osteogenesis.

In contrast to its effect on bone formation, the relationship between mechanical stress and bone resorption and how resorption sites are targeted in the skeleton are poorly understood. One recent proposal has been that osteocyte apoptosis associated with physical microdamage to the bone matrix may modulate the localized removal of bone (Noble *et al.*, 2003). The expression of collagenase-3 (MMP-13) in the osteocytes of alveolar bone adjacent to compression sites in a rat model of tooth movement suggests another potential mechanism (Takahashi *et al.*, 2003). The response of connective tissue cells to compressive mechanical strain is clearly an area that remains to be systematically investigated.

#### *Cytokines and tooth movement*

At a Biology of Tooth Movement conference held in 1986 Meikle *et al.* (1989) proposed that cytokines such as IL-1 produced locally by mechanically activated cells were responsible for mediating both the resorptive and formative phases of connective tissue remodelling. The immunolocalization of IL-1 $\beta$  in the periodontal tissues of cat canine teeth following the application of a tipping force



**Figure 11** Roles of receptor activator of nuclear factor  $\kappa$  B ligand (RANKL), osteoprotegerin (OPG), and M-CSF in the regulation of osteoclast maturation and function by osteoblasts/stromal cells. RANKL is found both as a transmembrane receptor and as a soluble cleaved form; OPG has been found only in secreted form. RANKL and OPG constitute a ligand–receptor system that directly regulates osteoclast differentiation. OPG acts as an inhibitor of osteoclastogenesis by competing with RANKL for the membrane receptor. M-CSF (CSF-1) acts directly on osteoclast precursor cells to control their proliferation and differentiation. Stimulators of bone resorption such as 1,25(OH) $_2$  vitamin D $_3$ , parathyroid hormone, and interleukin-1 increase osteoclast formation by stimulating the expression of RANKL by osteoblasts/stromal cells. (From Meikle, 2002.)

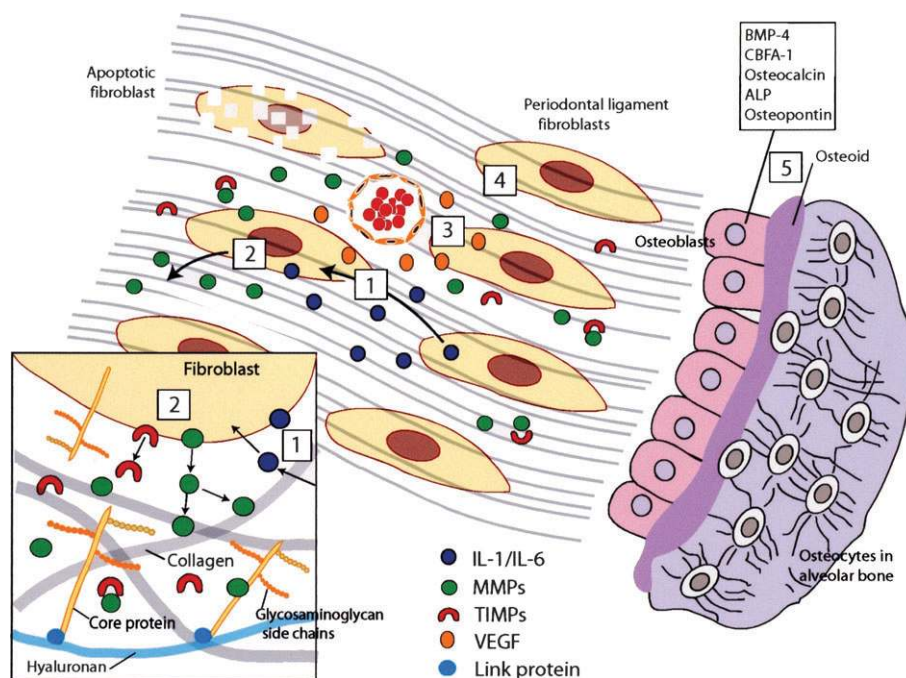
(Davidovitch *et al.*, 1988) provided the first experimental evidence in support of this hypothesis. Clinical studies have since shown that IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and epidermal growth factor are all elevated in gingival crevicular fluid collected from patients during the early phases of orthodontic tooth movement (Grieve *et al.*, 1994; Lowney *et al.*, 1995; Uematsu *et al.*, 1996). These findings have since been confirmed by numerous other investigations.

#### *The response of PDL cells to mechanical strain in vitro*

Cultured human PDL cells have been widely used to study the effects of tensile mechanical strain on gene expression at both message and protein levels, particularly for investigating intermediate- and late-acting genes. The results can sometimes be ambiguous, however, depending on the culture conditions, stress regimen (intermittent or cyclic versus continuous strain), and length of culture. For example, cyclic tension has been reported to both stimulate (Saito *et al.*, 1991; Shimizu *et al.*, 1994) and inhibit (Long *et al.*, 2001) IL-1 $\beta$  activity by PDL cells. While a continuous tensile strain applied to PDL cells stimulated MMP-1 (collagenase-1), MMP-2 (gelatinase-A), TIMP-1, and TIMP-2 expression (Bolcato-Bellemin *et al.*, 2000), centrifugation of PDL cells in culture flasks up-regulated mRNA levels for MMP-1, but had little or no effect on type

I collagen or TIMP expression (Redlich *et al.*, 2004). Cyclic stretching (15 per cent elongation) decreased ALP activity in PDL cells (Chiba and Mitani, 2004), but the synthesis of other matrix proteins such as type I collagen and fibronectin by PDL cells exposed to 5 per cent deformation was increased (Howard *et al.*, 1998). Also one cannot assume that data derived from one differentiated cell type is transferable to another; the synthesis of IL-10 was up-regulated by cyclic tension in human PDL cells (Long *et al.*, 2001) and down-regulated in murine osteoblasts (García-López *et al.*, 2005). From all the evidence available to date, however, it is possible to construct the hypothetical model in Figure 12, showing the response of the cells on the tension side of the PDL to orthodontic tooth movement.

To date, few researchers have utilized *in vitro* methods to investigate the response of either osteoblasts or PDL cells to compression forces. Kanzaki *et al.* (2002) found that culture media from compressed PDL fibroblasts stimulated osteoclastogenesis in peripheral blood mononuclear cell cultures and that RANKL mRNA expression was up-regulated in the cells. OPG expression in contrast remained unchanged. These data suggest that the PDL plays a pivotal role in osteoclast differentiation and function during orthodontic tooth movement. In another study, He *et al.* (2004) showed that PDL cells subjected to tensional and compressive forces were able to perceive two different



**Figure 12** Remodelling of the periodontium: tension side. In this hypothetical model periodontal ligament (PDL) fibroblasts under tensile strain synthesize cytokines such as interleukin-1 (IL-1) and IL-6 (1); IL-1 and IL-6 in turn stimulate matrix metalloproteinase (MMP) and inhibit tissue inhibitor of metalloproteinase (TIMP) synthesis by PDL cells via autocrine and paracrine mechanisms (2); vascular endothelial growth factor (VEGF) produced by mechanically activated fibroblasts promotes angiogenesis (3). Degradation of the extracellular matrix by MMPs facilitates cell proliferation and capillary growth; PDL cells (4), osteoblasts, and bone-lining cells (5) enter a biosynthetic phase with the synthesis of structural and other matrix molecules.

forms of mechanical stimuli and responded in a differential manner as regards ECM synthesis (type I collagen, fibronectin) and degradation (MMP-2, TIMP-2).

#### Molecular studies of tooth movement in the rat

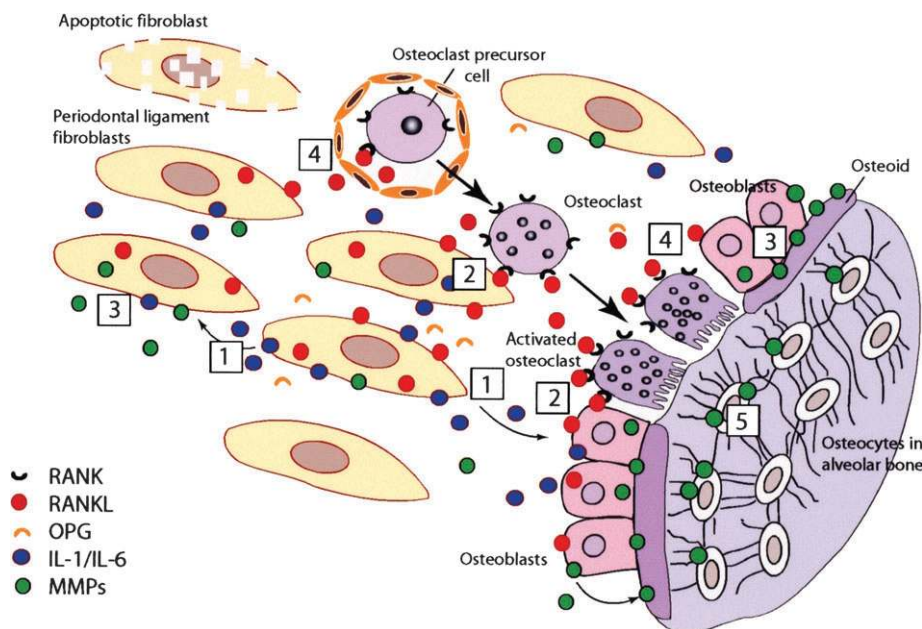
Connective tissue degradation and bone resorption involves the interaction of several differentiated cell types. *In vivo* methods are therefore essential if the cascade of molecular events responsible for the recruitment and activation of osteoclasts, as well as multinucleated giant cells, is to be fully understood.

The application of *in situ* hybridization techniques to tooth movement studies in the rat has proven to be particularly useful in this respect. The expression of IL-1 $\beta$  and IL-6 mRNAs (but not TNF- $\alpha$ ) has been shown to be up-regulated in both PDL cells and osteoblasts on the compression side (Alhashimi *et al.*, 2001), as is MMP-8 (collagenase-2) and MMP-13 (collagenase-3; Takahashi *et al.*, 2003). Both RANKL and OPG mRNAs are widely expressed in osteoblasts and PDL cells throughout the periodontal tissues (Ogasawara *et al.*, 2004), and following tooth movement using the Waldo method (Waldo and Rothblatt, 1954), positive signals for RANKL and RANK were detected in multinucleate osteoclasts at sites of active bone resorption. IL-1 $\beta$  and TNF- $\alpha$  expression was also identified in some osteoclasts. Figure 13 represents a

hypothetical model of the events involved in remodelling the periodontium on the 'compression' side during orthodontic tooth movement.

#### Is orthodontic tooth movement an inflammatory process?

The proposal that tooth movement involved the development of inflammation seems to have originated with a review paper by Storey (1973). In the 1950s, he published a series of papers on the histological changes produced by helical torsion springs applied to rodent (rat, guinea pig) and largomorph (rabbit) incisors (Storey, 1955a,b,c). The magnitude of the force applied to the teeth (25, 50, 250 g) was clearly excessive in this experimental model judging from the photomicrographs, and although a good deal of tissue destruction was evident, at no time was any reference made to the involvement of an inflammatory response. The demonstration that cytokines (frequently referred to in the literature as inflammatory mediators or pro-inflammatory cytokines), as well as neurotransmitters such as calcitonin gene-related peptide and substance P, are involved in remodelling the PDL has revived the concept that tooth movement is an inflammatory process (Davidovitch *et al.*, 1988; Davidovitch, 1995). This raises two questions: first, are cytokines unique to inflammation and second, to what



**Figure 13** Remodelling of the periodontium: compression side. In this hypothetical model periodontal ligament (PDL) cells under compressive strain synthesize interleukin-1 (IL-1) and IL-6 (1); IL-1 and IL-6 act in an autocrine and paracrine manner to up-regulate receptor activator of nuclear factor  $\kappa$  B ligand (RANKL) (2) and matrix metalloproteinase (MMP) (3) expression by PDL cells and osteoblasts. Osteoblast-derived MMPs degrade the non-mineralized surface osteoid layer of bone, while MMPs produced by PDL cells degrade their extracellular matrix; (4) RANKL stimulates the formation and function of osteoclasts from mononuclear precursor cells which access the bone surface and degrade the mineralized matrix; (5) deformation of the alveolar bone up-regulates MMPs expression by osteocytes adjacent to the bone surface.

extent can tooth movement be regarded as an inflammatory event?

The answer to the first question is no. The naming of cytokines has customarily been based on the biological activity by which they were originally identified. For example, before being sequenced, IL-1 was known by several names (lymphocyte-activating factor, endogenous pyrogen, catabolin, mononuclear cell factor) depending on the bioassay in which it was active and the research interests of the group that had identified it. The first cytokines were discovered in lymphocytes and monocytes through their role in mediating various aspects of the inflammatory and immune response and called lymphokines and monokines, respectively, by immunologists. Many, including IL-1 and TNF- $\alpha$  (cachectin), were eventually shown to be produced by numerous cell types including connective tissue cells and to be involved in normal physiological turnover and bone remodelling. Furthermore, both IL-6 (B cell growth factor-II, B cell stimulatory factor, interferon- $\beta$ 2, plasmacytoma growth factor) and IL-11 (adipogenesis inhibitory factor) have been shown to stimulate bone resorption and IL-10 (cytokine synthesis inhibitory factor) to inhibit bone resorption, a property not immediately obvious from their original names. Does that make bone remodelling an inflammatory process? It seems unlikely this is the case. Cytokines and PGs are not unique to inflammation; they are ubiquitous cell signalling molecules involved in numerous pathophysiological processes throughout the body. The statement of Tuncay (2004) that tooth movement is the end-product of an inflammatory response because it is diminished by NSAIDs merely shows that arachidonic acid metabolites are involved in PDL remodelling and their activity can be blocked by COX inhibitors.

The answer to the second question is more complex and depends on the definition of inflammation. Inflammation is commonly understood to be the localized host response to microbial infection or tissue damage. Under normal circumstances, tooth movement is a sterile process and the extent of any damage to the tissues will depend upon the magnitude of the applied force. Furthermore, tooth movement does not meet the four classical criteria for inflammation (redness, swelling, heat, and pain), perhaps apart from pain. In the article by Storey (1973), the question of inflammation in tooth movement is raised only in connection with the application of heavy forces. For example,

Those forces which exceed the bioplastic limit result in biodisruptive deformation with interruption of nutrition, ischaemia, and cell death as well as inflammation and rupture of connective tissues. Biodisruptive deformation is usually followed by the classic process of tissue repair (p 293). Or (p 304) ... heavy forces rapidly disrupt the

periodontal tissues and initiate an inflammatory process but, when the tooth stops moving, repair occurs; mitotic figures in fibroblasts are numerous, and within the first day or two a fine fibrin network fills the gap and cells and fibres bridge discontinuities in the tissue while macrophages remove cell debris. During this repair phase, vessels are no longer abnormally permeable ....

This description sounds remarkably like wound healing, a process composed of a continuous sequence of inflammation and repair designed to restore normal tissue continuity and function. Describing orthodontic tooth movement as an inflammatory process creates the impression that it is a pathological event. For the most part it is not, unless the clinician or animal investigator happens to be applying excessive forces to the teeth. If one wishes to employ a label to describe the tissue response to tooth movement, it is best regarded as an exaggerated form of normal physiological turnover combined with foci of tissue repair, particularly at compression sites where hyalinized tissue and adjacent bone and cementum are being remodelled.

## Conclusions

After 100 years, we have reasonably good understanding of the sequence of events involved in orthodontic tooth movement at the tissue and cellular levels on both the tensile and compression sides of the periodontium. At the molecular level, however, although significant progress has been made over the last 20 years, our knowledge remains far from complete. Both *in vivo* and *in vitro* methods have been widely used to investigate the response of cells to mechanical deformation and it is important to stress that the two approaches are complementary; data from *in vitro* model systems in which the mechanical stimulus applied to the cells can be carefully regulated (tension versus compression; intermittent versus continuous) should be correlated with *in vivo* data obtained from animal models.

Cell cultures have been widely used to investigate signal transduction mechanisms particularly in osteoblasts and, more recently, to study the expression of intermediate- and late-acting genes in both osteoblast and PDL cultures. Nevertheless, the focus to date has been on the effects of tensile strain. The development of instruments based on the vacuum operated apparatus described by Banes *et al.* (1985) designed to apply static or cyclic variable compression to cells *in vitro* should resolve this deficiency. Future investigations of gene expression involving PDL fibroblasts should also consider culturing the cells in a type I collagen gel matrix to create an artificial three-dimensional (3D) PDL. Unlike cells on two-dimensional surfaces which have a spread morphology, fibroblasts in 3D matrices develop an elongated or stellate form more closely resembling their shape *in vivo* which may influence signalling pathways and gene expression (DeMali *et al.*, 2003).



The molecular techniques of reverse transcription-polymerase chain reaction and *in situ* hybridization to detect gene expression in cell and tissue sections have revolutionized tooth movement studies, particularly in the rat, which despite some anatomical and physiological shortcomings (Ren *et al.*, 2004) remains the experimental animal of choice. In addition to detecting message levels, an expanding list of antibodies and enzyme-linked immunosorbent assays directed against human and animal proteins will facilitate their identification in tissue sections and/or culture supernatants. Although this technology has greatly simplified research for the clinical and laboratory investigator, one should not forget that the message is not always translated into protein, and the presence of a protein does not necessarily mean that it is biologically active. An MMP such as collagenase may be in a latent or active form or even complexed with TIMP.

The current evidence from these and other model systems suggests that downstream from the initial mechanotransduction event at focal adhesions which link the ECM to the cytoskeleton, connective tissue remodelling is mediated by a complex feedback mechanism involving the synthesis of osteotropic cytokines such as IL-1, IL-6, and RANKL by cells of the osteoblast and/or PDL fibroblast lineages. These in turn act in an autocrine/paracrine manner to regulate the expression of transcription factors, cytokines, growth regulatory molecules, and enzymes involved in the differentiation and function of mesenchymal and other cell types.

It would appear that Sandstedt and Oppenheim arrived at different conclusions regarding the tissue response to orthodontic tooth movement on the seemingly trivial difference of the plane of section—in Sandstedt's figures the tissue blocks were sectioned horizontally and in Oppenheim's vertically. Oppenheim (1930) was resistant to the idea that bone could be physically deformed but the pattern of the cellular response in his illustrations strongly suggests he was describing precisely that. It is perhaps ironic therefore that while Oppenheim is identified in many people's minds as the key figure in tooth movement research, Sandstedt's concepts dominate orthodontic theory today. The message for the contemporary investigator is that (1) a 3D perspective is essential for a complete understanding of events and (2) tooth movement involves two interrelated processes: the bending of alveolar bone and remodelling of the periodontal tissues.

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