

Seminar 12

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Evolving concepts in the mechanobiology of bone

Mechanical stimuli play an important role in the differentiation, growth and maintenance of skeletal tissues. It has been estimated that environmental factors such as physical activity and nutrition account for 20–40 percent of individual variation in bone mass, the remaining 60–80 percent being determined by genetic factors (Krall and Dawson-Hughes, 1993; Välimäki *et al.*, 1994). Mechanical forces in the form of tensile, compressive or shear stresses may be (1) growth-generated as in embryonic tissues with differential growth rates – the *Entwicklungsmechanik* or developmental mechanics of Wilhelm Roux; (2) the result of functional movement in articulations such as the temporomandibular joint and growing long bones; (3) the consequence of physical activity, including mastication; and (4) the result of external forces originating from man-made orthodontic and orthopaedic appliances.

An improved understanding the mechanisms by which mechanical stimuli are converted into biochemical events such as bone remodelling, is clearly important for the practice of orthodontics, orthopaedics and sports medicine based on sound biological and bioengineering principles. However, the borderland between biomechanics and cell biology is an intellectual minefield, and as anyone who has ever submitted a manuscript on the subject to an academic journal can probably confirm – commonality of purpose does not guarantee mutuality of comprehension.

The relationship between the form and function of bone

The first detailed study of the microscopic structure of bone was undertaken by the Spanish artist and anatomist Crisóstomo Martínez (c.1638–1694), commissioned by the University of Valencia, to create an atlas of anatomy that would enhance the prestige of the medical faculty. Martínez ‘the man who discovered trabecular bone,’ is thought to have started work on the atlas between 1672 and 1680 before moving to the *Académie des Sciences* in Paris, a recognized centre of excellence in anatomical studies at the time (Gomez, 2002; Valverde, 2009). By dissecting dry and fresh bones and using a simple microscope with an optical resolution of 0.7 µm, he was able to record for the first time the internal divisions of bone into an outer cortex of compact bone, and an inner trabecular network of cancellous bone in a series of accurate and beautiful engravings (Slide 3). Unfortunately, Martínez died in 1694 leaving just nineteen engraved plates (preserved in the Historical Archives of the City of Valencia) and his *Atlas of Osteology* was never published, except as a facsimile edition in 1964 edited by the medical historian López Piñero.

Slide 3. Plate X ‘metatarsus’ (circa 1690) in *El Atlas Anatómico de Crisóstomo Martínez. Grabador y Microscopista del siglo XVII*. The first detailed study of the microscopic structure of bone showing the cancellous and trabecular architecture. Edited by Jose Maria López Piñero, Valencia, 1964.

John Hunter and the femoral head

Martínez was first and foremost an artist and not trained in medicine. It was not until 1754 when John Hunter (1728–1793), building on the vital staining and implant methods of Belchier (1736) and Duhamel (1742), began his research at the anatomical school of his brother William in Covent Garden, London (Keith, 1918), that interest was drawn to the relationship between mechanical function and the structure of bone. Despite the efforts of the *Luftwaffe* during the summer of 1940, the Hunterian Museum in the Royal College of Surgeons of England still contains a number of Hunter’s original preparations that survived the Blitz, including a specimen showing the arrangement of the trabecular bone at the proximal end of the human femur, preserved in oil of turpentine (Slide 4).

Throughout the nineteenth century, the femoral head continued to be the focus of attempts to understand how the internal structure of bone was determined by functional mechanical loading. In 1838 while still a 20-year-old medical student at King's College Hospital, London, Frederick Oldfield Ward (1818–1877) published a small pocket-sized book entitled *Outlines of Human Osteology* (Dobson, 1949), in which he described not only the trabecular pattern of the proximal human femur, but also its functional significance (Slide 5). Twenty years later, Sir George Humphry (1820–1896), at the time Professor of Anatomy at the University of Cambridge published *A Treatise on the Human Skeleton* (Humphry, 1858), which contains a plate of several drawings, both in vertical and horizontal section, of the cancellous architecture of the femoral head (Slide 6). However, it was the collaboration between Georg Hermann von Meyer (1815–1892) and Karl Culmann (1821–1881) to explain bone structure, which remains the traditional starting point for discussions of the relationship between the form and function of bone.

The trajectorial theory of bone

In July 1866 at a gathering of naturalists in Zurich – the *Zürcher Naturforschende Gesellschaft* – von Meyer, Professor of Anatomy at the University of Zurich, delivered a lecture entitled *On the significance of bones as machine parts and the structure of cancellous bones* (Rüttimann, 1992), in which he proposed that the trabeculae in cancellous bone were laid down along the lines of maximal compressive and tensile stress. This suggested that both pressure and tension could behave as morphogenetic stimuli in bone (although tension was regarded as being more important), and the stress lines became known in anatomical theory as trajectories. Culmann, a pioneer of graphical methods in engineering (Culmann, 1866) was in the audience; he noted that von Meyer's anatomical drawings of the cancellous architecture of the femoral head closely resembled the trajectories an engineer would draw in a crane of the same extended form as the femur (a Fairbairn crane) and of homogeneous or solid structure (Slides 7, 8). The following year von Meyer published an article entitled *Die Architektur der Spongiosa* (1867) illustrating the stress trajectories of cancellous bone in the proximal femur and other bones, together with the mathematical calculations of Culmann's students for a Fairbairn crane (Slide 9).

Slide 9. This illustration (*Tafel XVIII*) showing that the trabeculae of cancellous bone were laid down according to specific stress trajectories in various bones, accompanied von Meyer's 1867 article. Also included (Fig. 10) is a drawing of the stress trajectories calculated by Culmann's students for a Fairbairn crane. (From von Meyer (1867). *Archiv für Anat und Phys*)

Julius Wolff and his law

Julius Wolff (1836–1902), an aspiring Berlin surgeon with an interest in orthopaedics read von Meyer's article, believing it to be “one of the most extraordinary discoveries of physiology” (Wolff, 1870), and arranged a visit to Zurich, where von Meyer showed Wolff his specimens. Wolff returned to Berlin and made a detailed examination of the trabecular patterns of various bones, although like his predecessors focusing largely on the proximal end of the human femur (*Oberschenkel*). His first papers included detailed illustrations, and the observation that when a bone became deformed, its internal structure was radically altered, but eventually adapted to its new function (Wolff, 1870; 1873; Slides 10, 11). These observations were gradually expanded into his *magnum opus* – *Das Gesetz der Transformation der Knochen* (*The Law of Bone Transformation*) published in 1892. In its final form what we know as Wolff's law eventually became defined as: “Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws.” Or to put it more simply, the structure of bone is morphologically adapted to its function (Slide 12).

Slide 11. Image 1 shows the forces and trajectories calculated by the students of Professor Culmann for a curved Fairbairn crane. Image 2: Architecture of the cancellous bone of the femoral head. The smaller drawings provide details of the mechanics of the crane and stress distributions in various structures. (From Wolff (1870). *Virchow's Arch Pathol Anat Physiol*)

What is surprising, is that for a man whose name became immortalized by one of the best-known eponyms in medicine, Wolff had a remarkably poor grasp of bone physiology. He believed that

bone grew interstitially, rejected the idea that bone could undergo resorption (something John Hunter had shown more than one hundred years earlier), and inexplicably given the supremacy of German biomedical science in the nineteenth century, had little understanding of cell theory or the function of bone cells (Keith, 1919). Given this background, as Dibberts (1992) has pointed out, it is obvious that for English translations of *Das Gesetz der Transformation der Knochen*, the contemporary term *Remodelling* (which includes resorption and formation) should not be substituted for *Transformation* – as in the case of the 1986 translation by Maquet and Furlong.

Objections to the trajectorial theory

The trajectorial theory was not without its critics, however, the most prominent being Triepel (1922). A difficulty of fundamental importance arose from the fact that an engineer in calculating the distribution of trajectories in a stressed body, assumes the body to be homogeneous in structure. Bone is clearly not structurally homogenous and in Triepel's opinion, the assumption that the trajectories in bone will be closely similar to an ideal homogeneous body was fundamentally flawed (since the primary literature is in German, the paper by Koch (1917) entitled *The Laws of Bone Architecture* is helpful).

In considering the various points of view, Murray concluded in *Bones. A Study of the Development and Structure of the Vertebrate Skeleton* (1936; pp 109–135), that while mechanical forces play a large part in the determination of bony structure, the trajectorial theory in its rigid form demands a degree of perfection that does not exist in Nature (unlike von Meyer, Wolff insisted the trajectories were orthogonal, conveniently ignoring the fact that trabeculae do not always cross each other at right angles). While such a difference of opinion can be regarded as a minor academic tiff, a more important difficulty is that Wolff's Law only takes into account the positive effects of mechanical loading, when it is evident that mechanically-induced strain can have negative as well as positive effects on bone mass (Slide 13).

Reaction of cortical bone to mechanical stimuli

The 1960s saw important advances in understanding the strain-dependent adaptation of bone to function by the introduction of *in vivo* models, in which carefully controlled external loads could be applied to bone (Slide 14). This approach was pioneered by Hěrt and co-workers in Czechoslovakia, who inserted paired 2.0–2.5 mm Kirschner wires into the right tibiae of rabbits; these emerged through the skin and were attached to an electromagnetic device that permitted approximation or distraction of the wires (Hěrt *et al.*, 1969; Hěrt *et al.*, 1971; Lišková and Hěrt, 1971).

In a classic series of experiments, they were able to first show that static loading or deformation was unable to stimulate bone formation (Hěrt *et al.*, 1969). In contrast, the application of intermittent forces 1–3 hours per day for up to 30 days, resulted in osteogenesis on both the periosteal and endosteal surfaces of the cortex (Slide 15), irrespective of whether they were exposed to what was perceived to be a tensile or compressive mechanical strain (Lišková and Hěrt, 1971). Unfortunately, in the aftermath of the Prague Spring of 1968, and the invasion of Czechoslovakia by the Soviet Union and the Warsaw Pact to halt the Dubček reforms, Hěrt was dismissed from his post at Charles University in Pilsen, and his research terminated; Lišková managed to escape to Canada. It was not until after the fall of the Iron Curtain in 1989 that Hěrt was reinstated.

Slide 15. Results of an experiment in which a tensile surface stress of 2.0–4.6 kPa/mm² was applied to the lateral (minus = tension), and a compressive surface stress of 2.2–5.0 kPa/mm² to the medial side (plus = compression) of the right tibia in four rabbits by means of Kirschner wires transfixing the bone; the left tibia served as control. Intravenous tetracycline was given four times during the 30 day time-course which labelled newly formed bone (red). Increased periosteal and endosteal bone formation has occurred on both the lateral and medial sides of the bone in the experimental tibiae compared to the contralateral controls. (Redrawn from Lišková and Hěrt (1971). *Folia Morphologica*)

The findings of Hěrt *et al.* that bone is insensitive to static strain and appears unable to distinguish between intermittent tensile and compressive strain were confirmed by Lanyon and co-workers, and further extended by relating mechanical strain magnitude to the structure and form of bone,

initially in sheep during locomotion (Lanyon and Baggott, 1976); and later with a functionally isolated, externally-loaded turkey ulna preparation (Lanyon and Rubin, 1984; Rubin and Lanyon, 1985). Mechanical strain (ϵ) is the amount of deformation of a body to an applied force. It is defined as the ratio of the change in length (new length minus the original length; L_2-L_1) divided by the original length (L_2-L_1/L_1) and has no units; because the magnitude of the strain is very small it is usually expressed as microstrain ($\mu\epsilon$) which is $\epsilon \times 10^{-6}$. In the turkey model, isolating the ulna from normal load bearing for 8 weeks was insufficient to maintain the normal osseous architecture of the cortical bone (Slide 16B), as was the case with daily loads of 100 consecutive 1 Hertz load cycles producing peak strains of 500 $\mu\epsilon$. When peak strains were increased to 1,000 $\mu\epsilon$, however, normal bone mass was maintained (Slide 16A), and if the strains exceeded 3,000 $\mu\epsilon$ the result was osteogenesis on both the periosteal and endosteal surfaces (Slide 16C).

Slide 16. The positive and negative effects of mechanical stimuli on bone mass are beautifully illustrated in these experiments in which turkey ulnas have been subjected to different bone strains. In (A) daily loading of 100 consecutive 1 Hertz load cycles producing peak strains of 1,000 $\mu\epsilon$, maintains normal osseous architecture. (B) Isolation from load bearing for 8 weeks. The cortex is thinned and osteopenic from incompletely filled secondary osteonal remodelling. (C) Isolated from natural load-bearing for 8 weeks, but subjected to 100 cycles per day of an externally applied load producing peak strains of 3,000 $\mu\epsilon$ around the bone's circumference. New bone formation has been induced on both the periosteal and endosteal surfaces. (Adapted from Lanyon (1984) *Calcified Tissue International*)

All connective tissues undergo continual synthesis and degradation of their extracellular matrices. In the adult skeleton, during normal physiological turnover there is a balance between the amount of bone resorbed by osteoclasts, and that formed by osteoblasts to maintain a constant bone mass (Frost, 1963). Bone resorption and bone formation are therefore said to be coupled, a process of renewing the skeleton while maintaining its structural integrity; embodied in the A-R-F (activation-resorption-formation) sequence of the bone remodelling cycle. The likely reasons for remodelling are maintenance of calcium homeostasis, prevention of the accumulation of old bone, adaptation of the skeleton to altered function, and the repair of microfractures. Mechanical loading stimulates rapid changes in periosteal gene expression, and there is abundant evidence to suggest that the osteogenic response of bone to increased mechanical loading is not dependent on a preliminary phase of bone resorption, but the result of direct activation of the osteoblastic cells lining the bone surfaces (Pead *et al.*, 1988; Chambers *et al.*, 1993; Raab-Cullen *et al.*, 1994; Chow *et al.*, 1998; Boyde, 2003), thereby uncoupling the bone remodelling cycle (Slide 17).

Nevertheless, in a study using the pin-fixation technique of Hert *et al* in rabbits, McDonald *et al.* (1994) found that mechanically-induced strain, while predominantly an osteogenic stimulus, also increased some areas of resorptive activity on the endosteal surface of the mid-shaft cortical bone, irrespective of the loading regimen.

Bone bending and stress-generated piezoelectrical effects in bone

Piezoelectricity is the electric charge that accumulates in certain solid materials in response to applied mechanical stress that enjoyed a brief period of interest in bone research during the 1960s. In bone, the amplitude of the electrical potentials generated were found to be dependent upon the rate and magnitude of bony deformation, while polarity was determined by the direction of bending (Bassett and Becker, 1962). When a bone is bent, increased concavity was associated with bone formation and electronegativity; and increasing convexity with bone resorption and electropositivity (Epker and Frost, 1965; Slide 18).

In other words, in orthopaedic theory compression results in bone formation, unlike the pressure-tension hypothesis of tooth movement in textbooks of orthodontics, where compression results in bone resorption. The problem with piezoelectricity as a regulator of bone metabolism, was the lack of specificity of action required to regulate the activity of osteoblasts and osteoclasts functioning in close proximity; moreover, similar results could be obtained with dead bone. As more became known about cell-cell and cell-matrix interactions, stress-generated electrical potentials became an irrelevant by-product of deformation – a physical phenomenon that provided a plausible explanation before growth factors, cytokines and other locally-produced

biochemical mediators enabling osteoblasts and osteoclasts communicate with each other were discovered in the 1980s (Slide 19).

The effect of reduced bone strains

The evidence from animal experimentation that increases in bone strains add to bone mass, while reduced bone strains lead to bone loss was supported by contemporaneous clinical studies of athletes (Nilsson and Westlin, 1971; Jacobson *et al.*, 1984), patients enduring prolonged bed rest (Donaldson *et al.*, 1970), and the effect of weightlessness on the skeleton during spaceflight (Jee *et al.*, 1983; Vico *et al.*, 1987; Wronski *et al.*, 1987; Dehority *et al.*, 1999). Stress-shielding and osteopenia resulting from the implantation of rigid metallic devices into bone also became a well-recognized complication of fracture fixation and joint arthroplasty in orthopaedic surgery (Woo *et al.*, 1976; Huiskes *et al.*, 1992; Glassman *et al.*, 2006; Uhthoff *et al.*, 2006). Animal studies of masticatory hypofunction have also shown that interference with normal levels of occlusal loading following a soft diet, or the insertion of bite-raising appliances is associated with changes in jaw morphology (Moore, 1965), as well as reduced levels of trabecular and cortical alveolar bone (Bresin *et al.*, 1999; Kunii *et al.*, 2008). (Slides 20, 21).

An additional difficulty in discussing mechanically-induced bone strains, the terms osteopenia and osteoporosis mean different things to different people, and are often used as synonyms. In this discussion, *osteopenia* is used to describe the physical sign of negative skeletal balance that is localized and reversible. *Osteoporosis* in contrast is a pathological condition resulting from post-menopausal or age-related estrogen deficiency, increased osteoclastic activity, and generalized bone loss, which is difficult if not impossible to reverse through physical exercise and/or bisphosphonates (Slide 22).

Harold Frost and the mechanostat

To describe this tissue-level regulatory negative feedback mechanism and add some clarity to the relationship between form and function in bone, the fertile brain of Harold Frost came up with the term *mechanostat* – a neologism derived from thermostat (Frost, 1987), the basic idea being that for each bone in the skeleton there is an adapted state within the boundaries of which normal bone mass is maintained (Slide 23). If the bone strains exceed a certain critical threshold ($> 1500 \mu\epsilon$) the result is bone formation, and if reduced below another threshold ($< 1000 \mu\epsilon$) will result in bone loss and osteopenia. Unfortunately, the terminology adopted by Frost (modelling for increases in bone mass; remodelling for bone loss) can be confusing – particularly for biologists and others for whom the definition of remodelling includes resorption *and* formation. It would have been preferable simply to refer to the responses as bone formation and bone resorption, the meaning of which most people understand.

Slide 23. The principle of the mechanostat (Frost, 1987). For each bone in the skeleton there is an adapted state within the boundaries of which normal bone mass is maintained. If the bone strains exceed a certain critical threshold ($> 2000\text{--}3000 \mu\epsilon$) the result is bone formation; reduction below another critical threshold value ($< 50\text{--}200 \mu\epsilon$) will lead to bone loss and osteopenia. (Image from TeamBone.Com)

There is little doubt that the principle of the mechanostat has proved valuable in describing the complexity of the skeletal response to mechanical loading, and has led to a wider appreciation of how bones adapt to their functional environment (Skerry, 2006). However, the mechanostat in its original conception was very prescriptive regarding the magnitude of the strains required to alter bone mass, encouraging the view that strain magnitudes are the same in all bones. This is clearly not the case – the strains experienced by long bones during physical activity will be considerably greater than those experienced by the bones of the calvaria. The strains experienced in different parts of the same bone will also vary considerably (Skerry, 2006). As in all biological systems variation is the key. There is also the intriguing question of the part played by the embryological origin of the bones of the skeleton, since mechanostat theory does not explain everything.

Difficulties with the mechanostat

Phenotypic differences exist between individual bones depending on their anatomical location, and it has been known for some time that calvarial and limb bones do not demonstrate the same

responses to dynamic mechanical strain (Slide 24). Rawlinson *et al.* (1995) recorded normal functional strains as low as 30 $\mu\epsilon$ on the rat parietal bone and found that unlike tibial osteoblasts, calvarial bone cells did not respond to dynamic strain with increased synthesis of prostanoids (PGE₂, PGI₂), or glucose 6-phosphate dehydrogenase. Direct strain measurements in a human volunteer further showed that in the skull, the highest strains recorded (200 $\mu\epsilon$) were 10-fold lower than for the tibia (Hillam *et al.*, 1996), levels that in the rest of the skeleton would lead to profound bone loss. So what is it about calvarial bone that makes it resistant to low levels of mechanical strain?

Not all bones are created equal

As discussed in detail in Seminar 1, the bones of the mammalian skeleton can be divided into two types based on their mode of development. (1) The bones of the craniofacial skeleton (apart from the posterior cranial base) are derived from the cephalic neural crest (Le Douarin, 1982; Couly *et al.*, 1993), and (2) the rest of the skeleton derived from mesoderm; in the case of the axial skeleton from the somitic or paraxial mesoderm, and the appendicular skeleton from lateral plate mesoderm (Slide 25).

In addition to ontogeny, neural crest and mesodermal bone have also been shown to be different in a number of other ways. Much has been made of the fact that the bones of the craniofacial skeleton are membrane bones – formed when mesenchymal stem cells condense and differentiate into osteoblasts without the intervention of a cartilaginous precursor, whereas most of the bones of the axial and appendicular skeleton develop by endochondral ossification from cartilaginous anlagen closely resembling the final form of the bone. There is no doubt that these differences are particularly important during skeletal growth as genetic forms of dwarfism such as achondroplasia readily demonstrate. Nevertheless, one must remember that although a bone may be classified as cartilaginous, much of the osseous tissue (particularly the growth in its circumference) will have been produced by perichondrial and periosteal osteogenesis, *i.e.* by an intramembranous process.

Concentrations of the growth factors IGF-II (insulin-like growth factor-II) and TGF- β (transforming growth factor- β) are significantly higher in human calvarial bone, than in iliac crest or vertebral bone (Finkelman *et al.*, 1994), and *in vitro* studies have shown that FGF-2 (fibroblast growth factor-2) and IGF-II expression is higher in mandibular compared to iliac crest osteoblasts, which also divide more slowly (Kasperk *et al.*, 1995). It has been suggested these differences account for the superiority of neural crest bone in grafting procedures (Hall, 1999).

Differences between neural crest and mesodermal bone in the heterogeneity of the enzymes produced by their osteoclasts have also been reported. Lysosomal cysteine proteinases (CPs) were clearly involved (Delaissé *et al.*, 1984; Hill *et al.*, 1994a), but doubt remained over the role of matrix metalloproteinases (MMPs) such as collagenase (MMP-1), in the acidic microenvironment of the subosteoclastic resorption zone. Contradictory data was seemingly resolved when Everts *et al.* (1999) reported that the osteoclasts of rabbit long bones depend on cathepsins B and K for resorption, whereas the activity of calvarial osteoclasts involves both MMPs and CPs. While having a certain neat symmetry about it, it does overlook as Thomas Henry Huxley would have put it, one nasty little fact – that the osteoclasts shown by Hill *et al.* (1994b) to produce MMPs and TIMP-1 using immunocytochemistry, and whose activity in the resorption pit assay was partially blocked by MMP inhibitors, came from long bones.

The difference, however, is a subtle one and depends on whether or not the cells are stimulated. In the study by Hill *et al.* (1994b), MMP inhibitors were unable to prevent resorption by unstimulated osteoclasts, but did produce a partial reduction when the cultures were stimulated by IL-1 α (interleukin-1 α). This requirement for femoral osteoclasts to be stimulated presumably accounts for the phenotype of pycnodysostosis, an autosomal recessive lysosomal disease characterized by dwarfism, clavicular dysplasia, skull deformity and osteopetrosis (Andren *et al.*, 1962; Maroteaux and Lamy, 1965); the disease, reputedly suffered by French artist Henri de Toulouse-Lautrec (1864–1901), caused by a mutation in the cathepsin K gene on chromosome 1q21 (Frey, 1995; Gelb *et al.*, 1996; Chen *et al.*, 2007). (Slide 26). Subsequent research with

cathepsin K knockout mice showed that while long bones developed osteopetrosis, calvarial bone was relatively unaffected (Cowen *et al.*, 2000).

Unfortunately, none of the above provides an answer to the original question: what is it about calvarial bone that makes it resistant to levels of mechanical strain that in the rest of the skeleton would lead to profound bone loss? It can't just be because calvarial bone is derived from the neural crest – the membrane bones of the jaws are also of neural crest cell origin, and do not show the same resistance to reduced mechanical loading (Slide 27).

Effects of orthodontic appliances on alveolar bone

Tooth movement studies to date have largely focussed on histological changes in the periodontal ligament (PDL) and supporting bone. But there is another important question that has received little if any attention that also needs to be addressed: What is the effect of the appliance itself on the metabolism and remodelling dynamics of the bone beyond the immediate tooth-supporting tissues? If nothing else, an orthodontic fixed appliance is a metallic device of varying rigidity, and in the light of the preceding discussion, might be expected to induce some degree of stress-shielding and osteopenia of the tooth-supporting bone (Slide 28). That statement is made with the benefit of hindsight, because in the course of a tooth movement study in the rat, we made the serendipitous discovery that a cross-arch expansion spring bonded to the maxillary molars of 6-week-old rats, resulted in a reduction in alveolar bone mass irrespective of whether the spring was active or passive (Slide 29; Milne *et al.*, 2009).

Slide 29. Appliance induced osteopenia. Stainless steel expansion spring (0.012 inch) delivering a force of 0.2N (\approx 20 gm) bonded to the first maxillary molar (M1) and second (M2) with self-etching primer and light activated composite resin. The expansile force produces an increase in the intermolar width averaging 0.25 mm by day 2 and 0.5 mm by day 8. An inactivated spring provided a positive control. (From Milne *et al.* (2009). *European Journal of Orthodontics*)

Unlike human teeth which exhibit physiological mesial drift rat molars drift distally and the distal alveolar walls are characterized by resorption; there is no distinct lamina dura and because of the small size of the jaws, secondary osteons are absent and marrow spaces are usually limited to the bone at the level of the apical third of the roots. Bone turnover is rapid; the duration of each remodelling cycle in the alveolar bone of adult rats is about 6 days (Vignery and Baron, 1980). In the first experimental series 6-week-old male Wistar rats received an injection of tetracycline 24 hours prior to sacrifice (Slide 30). Conventional histology showed that in the experimental group the PDL was thinned on the buccal side with foci of hyalinization and loss of normal tissue architecture (Slide 31). Measurements of IL-1 β levels in the serum by a rat-specific enzyme-linked immunosorbent assay (ELISA) were at the detection limit of the assay, and no differences in IL-6 levels by an ELISA (osteoblasts secrete IL-6 to stimulate osteoclast formation) were detected at any point in the time scale.

Slide 30. Uptake of tetracycline in control rats suggested a rapid turnover of bone in both the interradicular domain and the bone-PDL interface. In the experimental group the incorporation of tetracycline was much reduced even after 4 days, suggesting the appliance had produced a decline in the rate of bone turnover.

Slide 31. Representative images of the interradicular bone of M1 at the level of the middle third of the roots. The most significant change was in the appearance of the interradicular bone where there was clear evidence of osteopenia by day 8 particularly at the bone-PDL interface where there were numerous vascular communications with the PDL.

At sacrifice, blood was collected by cardiac puncture into glass tubes and allowed to coagulate for 30 min on ice, We then carried out alkaline phosphatase (ALP) and acid phosphatase (ACP) to determine whether the bone loss was due to a decrease in bone formation, or increase in bone resorption (Slide 32). Quantitative histomorphometry confirmed a significant reduction in alveolar bone after 8-days when passive springs were compared to controls (Slide 33).

Slide 32. Serum ALP was significantly decreased in both the experimental (by day 8) and sham (by day 4) compared to the zero-day controls. Serum ACP activity also showed a significant decrease in both the experimental and sham groups compared to zero-day controls at days 4 and 8.

Finite element analysis of tooth support in the rat

The skull of a 6-week-old control rat was scanned by X-ray microcomputed tomography using a SkyScan 1072 desktop micro-CT scanner (SkyScan, Aartelsaar, Belgium). Sections were taken at 35-micron intervals and a stack of 939 slices used to digitize the maxilla. Three-dimensional finite element analysis of the stresses generated in the bone following occlusal and orthodontic loading showed that the orthodontic force created a constant loading condition shielding some areas of bone from mechanical stress (Slide 34). Areas of low mechanical stimulation were coincident with sites of bone loss observed histologically, while bone mass was preserved in areas with higher levels of loading. These observations suggested the osteopenia had resulted from stress-shielding of the inter-radicular bone by the appliance, resulting in a reduction in occlusal loading below the critical threshold required for maintaining normal osseous architecture, and that the mechanostat model can be employed to explain, at least in part, the response of the bone to a fixed orthodontic appliance.

The FE method has been widely used in tooth movement studies to analyze the stresses and strains generated in the periodontium. In the rat FE analyses of orthodontic loading have shown high strain levels in the PDL and low strains in the bone (Bourauel *et al.*, 2000; Kawarizadeh *et al.*, 2004)), emphasizing the buffering capacity of the PDL. The FE analysis reported in Milne *et al.* (2009) suggested that the orthodontic appliance by disrupting the homeostatic balance of the alveolar bone, created competition between the mechanical stimuli that will govern the bone's adaptive reactions. While the masticatory load is much greater, it acts intermittently, and its effect on the bone appears to be muted by the damping effect of the PDL and the continuous action of the orthodontic appliance – in other words, the appliance changes the dynamics of the stimuli received by the bone.

Slide 34. Plots showing the distribution of the distortion energy (von Mises stresses in MPa) in the alveolar bone of the first and second maxillary molars. (Top) Following a masticatory occlusal load of 2N, the distortion energy is spread throughout the alveolar process affecting both the buccal and lingual plates and inter-radicular bone. (Bottom) During an orthodontic load of 0.2N produced by an expansion spring, the distortion energy is significantly less with little or no stress in comparable regions of the bone, correlating well with the histological data. (From Milne *et al.* (2009) *Eur J Orthod.* 3-dimensional FEA performed by Dr Ionut Ichim)

Edward Angle, bone strains, and orthodontic appliances

Experience tells me many orthodontists have difficulty bordering on outright denial, in accepting the implications of these findings. This is due to a long-standing doctrine dating back to the beginning of the last century, when it was thought orthodontic treatment had a positive effect on bone mass – ‘growing bone’ as Edward Angle (1855–1930) and many of his contemporaries believed, based on Julius Wolff's ‘Law of Bone Transformation,’ and the tooth movement research of Albin Oppenheim (See Seminar 13). At the time, orthodontic theory and practice was dominated by the nonextraction philosophy of Angle and his acolytes. Malocclusion of the teeth and jaws was regarded as being the consequence of inadequate bone growth, which could be corrected by alignment of the teeth – as Angle wrote in the Seventh Edition of *Malocclusion of the Teeth*: “If started young enough, the stimulating effects of orthodontic tooth movement and the establishment of normal occlusion would enhance growth of the jaws and alveolar process” (Angle, 1907). The view that malocclusion could be treated without extracting teeth by growing bone became the prevailing orthodoxy (Slide 35). This idea is still remarkably resilient amongst orthodontists, if my experience with the referees and Editor of the *American Journal of Orthodontics and Dentofacial Orthopaedics* is anything to go by; no amount of evidence could shake their belief in a 100-year-old myth. The idea that an orthodontic appliance might have a negative effect on bone mass was an alien concept – completely beyond their comprehension.

Slide 35. Thanks to Edward Hartley Angle's interpretation of Wolff's Law, it has been an article of faith amongst orthodontists for more than 100 years that orthodontic appliances can stimulate the growth of bone. To quote Angle in his 7th Edition (1907): “If started young enough, the stimulating effects of orthodontic tooth movement and the establishment of normal occlusion would enhance growth of the jaws and alveolar process” (pp 386–401). In other words, malocclusion could be treated without extractions by growing bone.

What does it mean?

- In accordance with mechanostat theory, these data suggest that an orthodontic appliance produces stress shielding of the inter-radicular bone, leading to osteopenia similar to that associated with prolonged bed rest and spaceflight – or the implantation of rigid metallic devices into bone following orthopaedic surgery for joint replacement and fracture fixation.
- Three-dimensional Finite Element analysis of the stresses generated in the bone, suggest that the orthodontic appliance created a constant loading condition shielding some areas of bone from mechanically-induced strain, that resulted in a reduction of occlusal loading below the critical threshold required to maintain normal osseous architecture.
- Instead of having a positive effect on bone mass, these data suggest an orthodontic appliance may exert unexpected negative side-effects on the tooth-supporting alveolar bone. Measurements of serum markers of bone metabolism indicate that the osteopenia is due to a reduction in bone formation, not an increase in resorption.
- This rather complicates how one interprets the effect of the appliance on bone metabolism in orthodontic patients – in addition to the alterations in bone remodelling associated with tooth movement mediated by the PDL and its potential buffering effect, there is the potential stress-shielding effect of the appliance itself to consider.
- These observations are also consistent with the findings of experimental masticatory hypofunction in rats, showing that reduced occlusal loading leads to a reduction in alveolar bone mass and bone mineral density.
- Finally, these findings highlight the importance of having positive as well as negative controls in experimental tooth movement studies, and recognizing that bone strains can have negative, as well as positive effects on bone mass.

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