

WOVEN BONE OVERVIEW: STRUCTURAL CLASSIFICATION BASED ON ITS INTEGRAL ROLE IN DEVELOPMENTAL, REPAIR AND PATHOLOGICAL BONE FORMATION THROUGHOUT VERTEBRATE GROUPS

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Abstract

Cortical bone development is characterised by initial formation of woven bone followed by deposition of lamellar bone on the woven scaffold. This occurs in normal bone formation as an integral obligate self-assembly pattern throughout all vertebrate groups, with specific temporal and spatial features. It also occurs in repair bone, modified by the biophysical/mechanical environment, and in pathological bone, modified by the specific disorder and its severity. Two spatially distinct osteoblast cell populations synthesise woven and lamellar bone: mesenchymal osteoblasts surround themselves circumferentially with collagen in a random array to form woven bone; surface osteoblasts align themselves in a linear array on the woven bone surface (or adjacent lamellar bone) to synthesise parallel-fibred lamellar bone. Four specific stages of woven bone formation are defined: stage I, early differentiation of pre-osteoblasts from undifferentiated mesenchymal cells; stage II, mesenchymal osteoblasts surrounding themselves in a 360° arc with randomly oriented matrix fibres; stage III, woven matrix acting as a scaffold on which surface osteoblasts begin to synthesise bone in parallel-fibred lamellar conformation; stage IV, progressive relative diminution of woven bone in the woven bone/lamellar bone complex. Stages II and IV are further subdivided (in a, b and c) by shifting cell area/matrix area and woven bone/lamellar bone relationships. The under-appreciated biological significance of woven bone is that it initiates formation *de novo* at sites of no previous bone. This information allows for targeted assessment of molecular-biophysical mechanisms underlying woven bone formation and their utilisation for initiating enhanced bone formation.

Keywords: Woven bone, lamellar bone, mesenchymal osteoblasts, surface osteoblasts, woven bone structural classification, repair bone, pathological bone.

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Introduction

Bone formation is characterised, at the tissue level and from the earliest embryological and foetal time periods, by an initial collagen matrix deposition in a randomly oriented pattern, referred to as woven bone, followed by collagen matrix deposition in a parallel orientation, referred to as lamellar bone. Woven to lamellar bone pattern, with lamellar bone synthesised on the woven bone scaffold, characterises normal cortical bone formation in all vertebrates. Not generally recognised are: i) the variable temporal and spatial patterns of woven bone replacement by lamellar bone across the entire vertebrate spectrum, including in extinct species, as defined by

palaeohistology; ii) the integral role played by the initial synthesis of woven bone followed by lamellar bone formation not only for normal bone but also in repair and pathological bone formation; iii) the specific stages underlying woven bone formation. There is a tendency to refer to this initial bone formation in its woven conformation as only a brief transitional phase and, then, to concentrate bone tissue studies almost exclusively on the subsequent lamellar bone. Much information can be gained by assessing the unappreciated crucial role of the woven bone stage throughout vertebrate bone formation.

References to woven bone in overviews of bone biology are brief, reflecting the general consideration of woven bone as a transient structure of little

significance (Buckwalter *et al.*, 1995) and poor quality (Marotti, 2010). Jaffe (1929) presented an excellent introduction to woven bone and Frost (1960) provided interesting differentiating features from lamellar bone. Descriptions of bone structure have concentrated on its well-ordered lamellar component. Havers, over 300 years ago, described bone as composed of a series of lamellae running along the longitudinal axis of the bone cortices; intracortical blood vessels were observed but there was no mention of a specific relationship of the vessels to the lamellae (Havers, 1691). It was not until the mid-1800s, with improved microscopy, that longitudinally oriented intracortical vessels surrounded by concentric lamellae were described and referred to

as Haversian systems (Todd and Bowman, 1845). That terminology for the characteristic orientation of human cortical bone lamellae was widely adopted and refined in light microscopy studies outlining matrix structure, cells and vessels of the bone cortices but no specific mention of precursor woven bone was made (Quekett, 1849a; Quekett, 1849b; Tomes and de Morgan, 1853). Continuing studies illustrated the lamellar structure of mature human bone with Haversian system predominance (Kölliker, 1853; Kölliker, 1886; von Ebner, 1875).

Gegenbauer named the cells actively synthesising bone as “osteoblasts” and specifically referred to the initial bone tissue deposited as “Wurzelstock” (root-stock), now referred to in English as woven or

Table 1a. Early descriptions of variable woven and lamellar bone orientation in vertebrate species.

Havers (1691)	Described the bone structure as a series of “strings” of particulate matter, referred to as lamellae, running along the longitudinal axis of bone cortices. “The bones...consist of several laminae, or thin plates, lying one within or under another...”; “The bony particles form continued threads or strings; which continuity gives the bones a rigidity. The course...of all the strings in the sides of a bone...(passes)... from one end towards the other; and wherever the laminae which they make are contiguous, they are parallel...and straight.”; “...the strings laid in a convenient order and number form the plates (laminae) and these plates make up the bone.” In human and animal bone, arteries and veins in foramina passing into marrow, bone pores and transverse and longitudinal passages were recognised. Havers did not specifically describe blood vessels running longitudinally within the circular collections of lamellae nor defined Haversian systems or osteons.
Todd and Bowman (1845)	Described longitudinally oriented intracortical vessels passing through the centre of several surrounding concentric lamellae. They were the first to refer to these structures in cortical bone as Haversian systems; “the arteries and veins usually occupy distinct Haversian canals”.
Tomes and De Morgan (1853)	Outlined matrix structure, cells and vessels of bone cortices; described formation of circular lamellae surrounding individual longitudinal vessels within the cortex and referred to these as “Haversian systems”.
Gegenbauer (1864, 1867)	Named the cells actively synthesising bone as “osteoblasts”, with initial bone tissue deposited as “Wurzelstock” (root-stock), referred to in English as woven or coarse-fibred bone.
von Ebner (1875), Kölliker (1886)	Recognised woven bone in their more detailed studies of bone formation; illustrated osteocyte cell shape and patterns in lamellar cortical bone.
Gebhardt (1906)	Described alternating fibre orientation in adjacent lamellae of human cortical bone.
Biedermann (1914)	Coined the term “osteon” (now used interchangeably with Haversian system): “...the unit dominated by bone blood vessels, this concentrically structured bone area forms the ontogenetic and mechanical single element (it could be called “the osteon”) of all higher developed vertebrate bones.”
Foote (1916)	Illustrated matrix and lacunar-canalicular patterns on cross-sections of femoral cortical bone throughout vertebrates (amphibians, reptiles, birds and mammals, human included). Studied human bones of various ages. No specific reference to woven bone. Defined intracortical structure as: <ul style="list-style-type: none"> i) lamellar, uniform lamellae encircling the entire bone diameter [subdivided into 3 distinct forms: uniformly concentric lamellae; two-fold division, with wide external and narrow internal lamellar rings in contact; three-fold division, with 3 concentric rings (external narrow, central wide and internal narrow)]; ii) laminar, a larger and more complex bone layer characterised by laminae separated from adjacent structures by vascular canals extending parallel to the long bone axis; iii) Haversian systems (osteons), main structural units, with circumferential lamellae surrounding a central longitudinal vessel, such as the one observed in higher mammals, including humans. Some specimens combined all types; more than 400 complete cross-section drawings of histological sections clarified accuracy of the descriptive terms.
Jaffe (1929)	Defined coarse-fibred primary bone and fine-fibred lamellar bone.
Weidenreich (1930)	Defined woven-fibred, parallel-fibred and lamellar bone. In woven-fibred bone, collagen fibres are arranged in a random fashion. In parallel-fibred bone, fibres run parallel to one another in the same direction. In lamellar bone, the fibres are arranged in layers (the lamellae), with the fibres in each layer having a specific orientation (different from adjacent layers).

Table 1b. More recent descriptions of tissue conformations in vertebrate cortical bone.

Gross (1934)	Commented extensively on “Faserknochen” (fibre bone) and stressed its more frequent occurrence in vertebrate cortices than in postnatal human. Also described the variable matrix structure patterns in vertebrate and fossil bone.
Enlow (1962), Enlow and Brown (1956, 1957, 1958)	Studied bone development throughout different vertebrate species and defined fossil bone structure in detail.
Smith (1960a, 1960b)	<p>Defined woven-fibred bone, primary osteons, secondary osteons, surface bone (outer circumferential lamellae) and interstitial bone. Described collagen fibre patterns in mammalian bone and in human secondary osteons.</p> <ul style="list-style-type: none"> • Woven bone: collagen fibres irregularly arranged, with no specific relation to adjacent vascular spaces. • Primary osteons: form around longitudinal vessels in these vascular spaces, with collagen fibres parallel to one another and sometimes segregated into lamellae. • Secondary osteons (Haversian systems): form after remodelling of primary osteons, involve synthesis of lamellae circumferentially arranged around longitudinally oriented vessels within the cortex. • Surface bone: specific lamellar structure (generally parallel-fibred bone) that forms in humans and many larger mammals, on periosteal and occasionally endosteal surfaces. • Interstitial bone: lamellar tissue remnants of primary and secondary osteons that persist as non-resorbed tissue in the constant remodelling process of growing and mature bone. <p>Primary cortical bone forms <i>de novo</i> along with woven bone in sites previously unossified, while secondary bone forms in sites where pre-existing primary bone has been resorbed; cement lines present around secondary osteons and absent within primary osteons.</p> <p>Human lamellae are arranged as alternating light and dark staining layers: longitudinal bone axis fibrils alternating with fibres running concentrically around a central vascular canal (transverse plane at right angles, orthogonally). Three types of fibrillar arrangements are present in secondary osteons: type I (described above), with alternation of lamellae with approximately equal concentration of longitudinal and circumferential fibres in adjacent lamellae; type II, similar alternation of lamellae, with disproportionate unequal fibril concentrations; type III, lamellation much less apparent, with little evidence of concentric layers.</p> <p>Documented i) continuous synthesis of periosteal woven bone and its persistence postnatally in many animal species, with cortical growth (fibrolamellar bone); ii) rapid disappearance of woven bone in humans after birth as Haversian system formation predominates (cortical secondary osteons); iii) subperiosteal accretion in many vertebrates is a repetitive process until skeletal maturity, with cycles of woven bone and primary osteon formation explaining the residual woven linear accumulations persisting in large mammalian and other vertebrate species.</p>
Cooper <i>et al.</i> (1966)	Presented a detailed assessment of the osteon morphology by transmission electron microscopy.
De Ricqlès (1975, 1976)	Refined the practice of paleohistology, introducing a classification and detailed descriptions of diverse cortical bone tissue conformations throughout vertebrate fossils, that became the standard for descriptive studies. Dense accumulations of Haversian bone, while being the common pattern in human cortices, are the exception since other vertebrates, fossil and living, have wide structural diversity. Classification pointed out persistence of fibrolamellar bone (woven bone surrounded by parallel lamellar fibres) in developing postnatal and mature animals, along with primary, but far fewer secondary, osteons. De Ricqlès and collaborators continued to assess vast numbers of species in fossil and living vertebrate histology (Francillon-Vieillot <i>et al.</i> , 1990; de Ricqlès <i>et al.</i> , 2016).
Giraud-Guille (1988), Giraud-Guille <i>et al.</i> (2003)	Working at the physical-chemical and ultrastructural level, identified the “twisted plywood” pattern of collagen deposition in lamellar bone and further characterised the concept of collagen matrix conformation as forming by self-assembly.

coarse-fibred bone (Gegenbaur, 1864; Gegenbaur, 1867). The formation, structure and continuous remodelling of bone at the tissue level have been the subject of detailed investigation: Jaffe defining coarse-fibred primary bone and fine-fibred lamellar bone; Weidenreich defining woven-fibred bone, parallel-fibred bone and lamellar bone; Smith defining woven-fibred bone, primary osteons, secondary osteons, surface bone and interstitial bone (Jaffe, 1929; Smith, 1960a; Smith, 1960b; Weidenreich, 1930). Table 1a,b detail the developing understanding of woven and lamellar bone in cortical bone formation.

There are several patterns of lamellar bone structure in vertebrate species. Foote, defining lamellar, laminar and Haversian system patterns of bone deposition, described: i) uniform lamellae encircling the entire diameter of the bone; ii) a more complex pattern characterised by laminae separated from adjacent structures by vascular canals; iii) multiple Haversian systems (osteons) in higher mammals, including humans (Foote, 1916). These differing patterns of lamellar bone organisation throughout vertebrate groups continue to undergo assessment, especially by palaeohistologists. The

matter is pertinent to a discussion about woven bone since cortical lamellar bone made strictly of Haversian systems (osteons) is primarily restricted to humans and some large mammals. Most species show a persistence of fibrolamellar bone (where the fibro component is woven bone) due to repeated cycles of periosteal bone formation, where woven bone is repetitively synthesised after lamellar bone deposition rather than undergoing full resorption and complete secondary osteonal structuring. Differences in vertebrate cortical bone microstructure in multiple species and the variable terminology describing it are summarised in Table 2 and 3a,b.

The shape and orientation of osteoblasts and osteocytes and their cell processes change as extracellular matrices pass from woven to lamellar conformations. Developmental changes in the lacunar-canalicular system (housing cells and their processes, respectively) were identified early in the microscopical study of bone (Bidder, 1906; Gebhardt, 1906; Gegenbauer, 1864; Gegenbauer, 1867; Kölliker, 1853; Quekett, 1849a; Quekett, 1849b; Tomes and de Morgan, 1853; von Ebner, 1875; Weidenreich, 1930). Osteoblast and osteocyte cell processes in canaliculi link adjacent cells through gap junctions (Burger and Klein-Nuland, 1999; Palumbo *et al.*, 1990; Shapiro, 1988; Shapiro *et al.*, 1995; Shapiro, 1997). The lacunar-canalicular system has structural and biomechanical implications (Currey, 2001) and serves as an accurate marker of cell activity in histological and palaeohistological assessments. Histological characteristics of woven and lamellar bone are detailed in Table 4.

Osteoblasts synthesising woven and lamellar bone are referred to using different terms to reflect their different modes of action. The osteoblast of woven bone is referred to as a mesenchymal osteoblast (MOBL), which surrounds itself in a 360° arc with initially formed woven bone with randomly arrayed collagen fibres, while the osteoblast of lamellar bone is referred to as a surface osteoblast (SOBL), since it aligns in a linear array on the surface of woven bone to synthesise parallel-fibred lamellar bone (Shapiro, 1988; Shapiro, 2008; Shapiro, 2016). While all osteoblasts are considered mesenchymal in lineage, the term MOBL was chosen to indicate initial or *de novo* bone formation in a cellular mass where no bone previously existed, as would occur in the mesenchyme of classical embryological descriptive terminology. Marotti and colleagues have referred to woven bone formation as static osteogenesis and lamellar bone formation as dynamic osteogenesis (Ferretti *et al.*, 2002; Marotti, 2010). The term static is used in their categorisation referring to the synthesis of woven bone occurring by differentiating cells surrounding themselves with matrix at a place where they are already present (hence static). The term dynamic is used to imply movement of cells to position themselves along bone surfaces where they synthesise lamellar bone. The cell population defined as MOBLs represents static osteoblasts while SOBLs represents dynamic osteoblasts. The terminology describing osteoblast synthesis of woven and lamellar bone formation is reviewed in Table 5a,b.

The present report outlines the deposition and structure of bone matrix from initial woven to eventual

Table 2. Differences between cortical bone structure in humans and other vertebrate species.

Primary and secondary osteons	Primary osteons form initially around blood vessels in woven bone; lamellar bone is laid down circumferentially surrounding a central vessel to form the osteon. Secondary osteons form following resorption of pre-existing bone and are distinguished structurally by surrounding dense staining cement lines. Primary osteons differ from secondary osteons as there is no tissue resorption accompanying their formation and no cement lines mark their extent.
Human foetal and post-natal bone	Study of humeral, femoral and tibial cortices show that: i) initially, early foetal woven bone synthesised by inner osteogenic layer of periosteum forms cortical tissue; ii) then, parallel-fibred lamellar bone is deposited on foetal woven bone cores as early as 4-4 ½ months, as shown by polarised-light microscopy; iii) it is not accurate to consider that human foetal cortical bone is invariably all woven; late foetal and early post-natal bone is progressively replaced by lamellar bone in primary osteons and, then, in secondary Haversian system osteons; iv) by 2-3 years after birth (other than at periosteal surfaces) all cortical bone becomes lamellar, arranged into dense Haversian systems.
Other vertebrate species (including many large mammals)	In other vertebrate species i) woven bone is continually synthesised by the inner layer of the periosteum pre- and postnatally and much of it persists until skeletal maturity; ii) woven bone recurrent production and persistence in successive layers as fibrolamellar bone is a prominent structural feature of the cortices; iii) fibrolamellar bone is structured in varying conformations such as longitudinal, plexiform, reticular and radial along with some primary osteons; iv) secondary osteons form in some species but rarely predominate.
Significant differences between structure of cortical bone in humans and other vertebrate species	There are significant differences between the structure of cortical bone in humans and other vertebrate species. Human bone, where almost the entire cortex has a predominantly secondary osteonal (Haversian system) structure with no woven bone after the first few years of life, shows considerable structural differences from most vertebrate species, including even some large mammals, demonstrating persistence of woven bone on which lamellar bone is deposited in plexiform and reticular patterns throughout the post-natal growth period.

Table 3a. Terminology for woven and lamellar collagen deposition in vertebrate cortical bone.

Classification of de Ricqlès (1975) for cortical bone tissue	Primary bone tissue: i) fibrous (woven) bone – avascular or vascular fibrous bone; ii) lamellar-zonal bone – avascular or vascular lamellar, periosteal lamellar with primary osteons; iii) fibrolamellar complex bone – fibrous bone with primary osteons. Secondary bone tissue: secondary osteons (Haversian systems) and non-Haversian lamellar bone (further descriptions include lamellar or parallel-fibred non-vascular bone, lamellar or parallel-fibred simple vascular bone, vascular canal surrounded by concentric bone lamellae or parallel-fibred laminae = lamellar primary osteon).
Descriptive terms for histology of bone cortex	A simpler terminology, increasingly found in many vertebrate studies, describes bone matrix fibrillar pattern orientation as: i) fibrous or woven-fibred; ii) parallel-fibred, composed of an orderly arrangement of closely packed fibres running parallel to one another; iii) lamellar, with alternating spatial orientation of the fibres of successive layers. In lamellar bone, the predominant fibre direction in the alternating layers is described as either an orthogonal (at right angles to one another) or twisted plywood arrangement with differing rotations.
Fibrolamellar bone or fibrolamellar complex	Fibrolamellar bone (fibrolamellar complex) forms the main structural unit of most cortical bones; it is composed of woven bone (matrix tissue without orientation) originating from the periosteum that forms a scaffold surrounded by primary osteons within lamellar or parallel-fibred bone. Thus, the tissue complex has a mixed structure and often dense vascularisation where the woven fibrous bone matrix combines with lamellar bone matrix forming intracortical primary osteons. Two additional terms (referred to above in de Ricqlès system) describe primary bone tissue synthesis, where the periodic growth layers show primary bone tissue disposed as i) lamellar-zonal bone, where the zones are composed of lamellar or parallel-fibred bone matrix (generally in external cortical layers); ii) fibrolamellar-zonal bone, where zones are composed of woven matrix with abundant primary osteons that tend to compose the bulk of the innermost larger bones.
Vessel orientation	Vessel orientation directs patterns of surrounding matrix deposition. Vascular orientation of the vessels and the pattern of bone formed can be longitudinal (vascular canals run parallel to the long axis of the bone), radial (vascular canals radiate from the marrow and periosteum and extend partially across the cortex, penetrating from the bone periphery at right angles to the long axis), plexiform (vascular canals organised into a regular well defined plexus linking adjacent layers with transverse bridges) and reticular (vascular canals disorganised, irregular and primarily oblique) (Currey, 1960; Enlow, 1962).
Three-dimensional structure of fibrolamellar bone	Much animal cortical bone is composed by repetitive layer synthesis of composites of woven and lamellar bone (fibrolamellar), with the pattern dictated by the matrix relationship to the vasculature. Four types are defined: laminar bone tissue, composed of fibrolamellar complexes of woven bone with primary osteons with a circumferential orientation of vascular canals; plexiform bone tissue, where the laminar bone has differing vascular orientations – longitudinal canals connected by radial and circular canals; reticular bone tissue, with numerous primary osteons with oblique vascular canals and overall irregular anastomoses; radial bone tissue, where the numerous primary osteons are radially oriented.

Table 3b. Proposed revisions to descriptive terminology for vertebrate cortical bone.

Fibrolamellar to be abandoned	The woven bone component of cortical bone was far less extensive than previously thought. Much of what had been considered woven bone appeared to be parallel-fibred (“non-lamellar”) arrayed in differing planes (from lamellar bone) as determined by polarised-light microscopy and multiplanar sectioning. Fast growing, highly vascularised composite bone tissue of woven bone and lamellar bone was referred to as laminar to plexiform primary bone (based on vascular organisation).
HOPB proposed	This term was proposed to define combined lamellar tissue (with primary osteons) and parallel-fibred tissue (referred to as non-lamellar). HOPB refers to bone in terms of its development, with woven bone produced by static osteoblasts (mesenchymal osteoblasts) and lamellar/parallel-fibred bone by dynamic osteoblasts (surface osteoblasts) (Stein and Prondvai, 2014).
Fibrolamellar complex term maintained (restricted role); new term, woven-parallel complex, added	The term fibrolamellar complex has been maintained (in a restricted role) and a new term, woven-parallel complex, added to incorporate early development. Thus, the term woven-parallel complex describes a broad range of bone-forming tissue and the term fibrolamellar complex is maintained as a subset of woven-parallel complex describing densely vascularised primary periosteal tissues (cortical bone) where woven/fibrous bone forms between the vascular canals and is then surrounded by lamellar or parallel-fibred bone (HOPB appeared to be useful to explain findings rather than serving as a separate classification term) (Prondvai <i>et al.</i> , 2014).
Correlation of concepts	The woven scaffold represents the fibrous component derived from static/mesenchymal osteoblasts and the lamellar component refers to both lamellar and non-lamellar (parallel-fibred) bone derived from dynamic/surface osteoblasts.

Table 4. Structural characteristics of woven and lamellar bone.

Matrix conformation	Woven bone has randomly arrayed collagen fibres; lamellar bone has fibres aligned in a parallel array.
Polarised light transmission	Woven bone is isotropic when examined by polarised-light microscopy, not transmitting light regardless of the plane of observation; lamellar bone is anisotropic, transmitting light that is parallel to the two parallel polarisers, due to the bone fibres being in a parallel array.
Osteoblast/osteocyte cell shape, number and position	Woven bone osteoblasts are round to oval, present in larger numbers (than cells of lamellar bone) per unit area and embedded within the collagenous matrix that surrounds them in a 360° arc. Lamellar bone osteoblasts are aligned on the surface of woven bone, deposit bone only on the surface they cover, touch one another and communicate through gap junctions and, generally, are only one cell-layer thick. Lamellar bone osteocytes are elongated and elliptical in shape, less numerous per unit area than woven osteoblasts and have their long axes parallel to the lamellae. Woven bone cells are referred to as osteoblasts when surrounded by a randomly oriented loosely packed collagen matrix since they continue to synthesise matrix; they become woven bone osteocytes when the woven bone is encased by lamellar bone.
Orientation of the lacunar-canalicular system	Woven bone osteoblasts/osteocytes are present randomly in round to oval lacunae, irregularly positioned throughout the woven bone and their canaliculi radiate in all directions from the cell surface circumferentially to course through the matrix in irregular pathways. Lamellar bone osteocytes are present in elliptical elongated lacunae that run parallel to the lamellae and their cell processes lie in canaliculi perpendicular to the walls of the lacunae.
Density and orientation of bone vascularity	Woven bone formation occurs in close relationship to vascularity but with no specific pattern. Lamellar bone formation also occurs in specific relationship to vascularity but Haversian system bone (osteons) forms circumferentially around longitudinal intracortical vessels as surface osteoblasts deposit lamellar bone progressively closing in on the vessel. Other vessel patterns are transverse (radial) in humans through Volkmann's canals and oblique in other species, as plexiform or reticular bone.
Rate and position of matrix synthesis	Woven bone is synthesised rapidly and <i>de novo</i> at sites where no bone tissue is present; lamellar bone is synthesised in a slower fashion and only on a pre-existing scaffold (of woven bone, lamellar bone or calcified cartilage).

lamellar patterns based on the authors' observations and investigations, a detailed literature review on matrix conformation in varying circumstances and palaeohistological studies documenting woven and lamellar bone structure in many vertebrate species.

Materials and Methods

Tissues examined

Normal bone, diaphyseal and metaphyseal femur and tibia from i) embryonic and postnatal mouse, rabbit and pig and ii) foetal, new-born, childhood and adult human.

Repair bone from i) skeletally mature rabbit, 2.4 mm diameter full-thickness cortical diaphyseal defects (Shapiro, 1988); ii) chicken, oval 10 × 3 mm full-thickness cortical diaphyseal defects (Glimcher *et al.*, 1980); iii) rabbit fractured-femur endochondral bone repair with external callus (Shapiro, 2008; Shapiro, 2016); iv) distraction osteogenesis rabbit tibial lengthening procedure (Shapiro, 2016; Shapiro *et al.*, 1992); v) full-thickness articular cartilage defects into subchondral bone in skeletally mature rabbit distal femur (Shapiro *et al.*, 1993); vi) transphyseal defects in skeletally immature rabbit proximal tibia (Jaramillo *et al.*, 1990); vii) piglet femoral head osteonecrosis secondary to ischemia (avascular necrosis) induced by intracapsular circumferential

ligatures around femoral neck base (Shapiro *et al.*, 2009).

Pathological human bone from osteogenesis imperfecta (femoral and tibial bone from 30 patients with lethal perinatal, progressively deforming and autosomal dominant variants) [Maguire *et al.* (2011) Osteogenesis imperfecta bone: correlation of histologic structure and radiographic appearance. American Academy of Orthopaedic Surgeons annual meeting, San Diego, CA, USA; Shapiro, 2016; Shapiro and Flynn (2005) Variable structure of osteogenesis imperfecta bone. *J Bone Miner Res* **20** Suppl 1: S175; Van Dijk and Silence, 2014] and osteosarcoma (distal femoral and proximal tibial metaphyseal tissue from 10 adolescent patients) (Shapiro, 1983; Shapiro and Eyre, 1982).

Light microscopy and polarised-light microscopy

Tissues were decalcified in 10 % formic acid and processed for light-microscope examination using either paraffin-wax-embedding (sections stained with haematoxylin and eosin or safranin O-fast green) or plastic-embedding (JB4 plastic, Polysciences, Warrington, PA, USA) (sections stained with 1 % toluidine blue). Light microscopy examination was performed using an Olympus BX50 microscope with digital camera. Tissue sections were examined by polarised-light microscopy (Olympus BX50 microscope). Matrix polarisation was documented

Table 5a. Terminology for osteoblast synthesis of woven and lamellar bone.

Osteoblast terminology	Shapiro (1988, 2008): MOBLs synthesise woven bone; SOBLs synthesise lamellar bone. Ferretti <i>et al.</i> , (2002); Marotti (2010): static osteogenesis leads to woven bone formation; dynamic osteogenesis to lamellar bone formation.
Early observation of the phenomenon	Knaggs (1925) recognised that intramembranous bone formation involves two different cell populations. Discussing pathological bone formation in osteitis deformans (Paget's Disease), Knaggs observed that, once original bone disappear, there is "the substitution of a vascular connective tissue (osteogenic tissue) in place of it and its intertrabecular marrow and the formation of new bone from this connective tissue, in the first instance always by metaplasia.....The term 'metaplasia' is used here to distinguish bone formation direct from the connective tissue from that which is associated with rows of osteoblasts."
Derivation of the terms MOBL and SOBL	While all osteoblasts are mesenchymal in lineage, the term MOBL was chosen to indicate initial or <i>de novo</i> bone formation within a cellular mass (woven bone) where no bone previously existed, as would occur in the mesenchyme of classical embryological terminology. The osteoblast of lamellar bone is referred to as SOBL since it aligns in a linear array on the surface of woven bone (or adjacent lamellar bone) to synthesise parallel-fibred lamellar bone.
Derivation of the terms static and dynamic osteogenesis	The term static refers to synthesis of woven bone (static osteogenesis) occurring by differentiating cells that surround themselves with a matrix at a place where they are already present (hence static). The term dynamic is used to imply movement of the cells to position themselves along bone surfaces where they synthesise lamellar bone (dynamic osteogenesis).
Mesenchymal cell	Maximow (1927) recognised and defined the mesenchymal cell as a precursor cell to connective and haematopoietic tissues and referred to it as a stem cell. Weiss and Garber (1952) defined the changing shapes of the mesenchymal cell in development. Kfoury and Scadden (2015) outlined molecular components of its contributions to forming connective and haematopoietic tissue.
Value of mesenchymal/surface terminology	Mesenchymal and surface are descriptive positional terms referring to osteoblasts. The first defines an osteoblast emerging from an undifferentiated mesenchymal tissue mass; the second, an osteoblast positioned in a linear array on a surface scaffold. Both define active processes requiring cell migration, movement and differentiation from the mesenchymal tissue mass at different times and positions. Bone formation <i>de novo</i> in the woven conformation (mesenchymal osteoblasts) is active and rapid; lamellar bone formation (surface osteoblasts) is a slower orderly process on the scaffold. The terms static and dynamic imply levels of activity that do not accurately reflect actual cell functions. The term static is inaccurate since these cells actively migrate to and differentiate at their functional sites and demonstrate strong synthetic force making bone <i>de novo</i> . Those cells are extremely active, forming bone faster, in a more dynamic fashion, than the slower and better oriented actions of the surface osteoblasts. Once a woven bone scaffold forms, cells already present switch to unidirectional surface synthesis from multidirectional pericellular synthesis.

Table 5b. Description of cells in woven and lamellar bone.

Lamellar bone	There is a clear distinction between lamellar bone osteoblasts and osteocytes. Osteoblasts are cells that are actively synthesising bone matrix. Osteoblasts synthesising lamellar bone are aligned in a linear array (often only one cell in depth), lie on a firm scaffold surface, deposit collagen in a unidirectional fashion, extruding it only from that part of the cell lying against the scaffold surface, and deposit collagen matrix with the fibres in a parallel array. These cells are referred to as SOBLs. Osteocytes are living bone cells no longer actively synthesising bone and surrounded by a mineralised matrix. Osteocytes of lamellar bone are elongated elliptical-shaped cells, have long axes that are parallel to the lamellae within which they lie and are embedded in a matrix that mineralises with lamellation.
Woven bone	The distinction between woven bone osteoblasts and osteocytes is not as clear as for lamellar bone. Osteoblasts are cells that are actively synthesising bone matrix. Osteoblasts synthesising woven bone are randomly positioned, lie within mesenchymal tissue, are round to oval, are densely packed initially with little surrounding matrix, deposit collagen in a 360° radius and become embedded in progressively increasing amounts of matrix whose collagen fibres are in random (woven) array. These cells are referred to as MOBLs.
When MOBLs can be considered as mesenchymal osteocytes	The structural identification of cells as MOBLs is more prolonged and not as rigidly defined as for SOBLs. This is because MOBLs are actively synthesising bone matrix (the defining term for an osteoblast) and depositing it circumferentially around the cell over a time from when there is very little matrix surrounding the cell until there is a relatively large amount of matrix surrounding it. There are woven bone osteocytes but (unlike in lamellar bone) a cell surrounded by matrix cannot serve as the definition of a woven bone osteocyte since it is still synthesizing tissue. MOBLs can be considered as no longer actively synthesising woven bone when the woven bone accumulation is lined by SOBLs and lamellar bone. Then, the woven bone cells are referred to as woven bone osteocytes.

with a light microscopy photomicrograph followed by a polarised-light microscopy view of the same section in the same orientation.

Light microscopy histomorphometry

Cell area/total area ratios in woven and lamellar bone

Histomorphometry quantified the cell area/total area ratios of woven and lamellar bone. This ratio served as a cell/matrix assessment reflecting the relative amount of matrix synthesised by a cell per unit area. Quantitation was performed on normal human bone from foetal, neonatal, childhood and adult specimens and on osteogenesis imperfecta bone from three main variants (lethal perinatal, progressive-deforming and autosomal dominant). Cells and types of matrix associated were outlined and cell area/total area ratios determined using the Zeiss Interactive Digital Analysis System (Zidas).

Length/width osteocyte and osteoblast ratios in woven and lamellar bone

The ratio of length to width was measured in lamellar bone osteocytes and woven bone osteoblasts/osteocytes in normal rabbit cortical bone and in rabbit repair bone from 2.4 mm cortical diaphyseal defects (Shapiro, 1988).

Transmission electron microscopy (TEM)

Tissues examined by TEM included new-born mouse femoral cortical bone and femoral and tibial bone from adolescent patients with osteogenesis imperfecta undergoing osteotomy. Cortical bone was processed by immediate sectioning into small fragments of approximately 1 × 1 × 1 mm on a cold glass surface (over ice) with tissues bathed with droplets of 1 % glutaraldehyde. The tissues were fixed using two parts osmium tetroxide to one part glutaraldehyde in 0.1 M cacodylate buffer at 0 °C for 3 h. Following fixation, bone was decalcified with 7.5 % ethylenediaminetetraacetic acid (EDTA) (pH 8.3) in 2.5% glutaraldehyde for 24 h when it was soft. The tissues were postfixed for 3 h in 1 % osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in increasing concentrations of alcohol, then placed in a 1 : 1 propylene oxide-Epon mixture for 1 week. Tissues were embedded in EPON 812 resin (3A:7B) (E.F. Fullam, Inc, Latham NY, USA), sectioned at 1 µm thickness, stained with 1 % toluidine blue for light microscopy examination and, then, trimmed and sectioned at 60 nm thickness (Ultratome III, LKB Instruments, Stockholm, Sweden) for TEM examination after staining with 3 % lead citrate (~ 30 min) and 0.5 % uranyl acetate (~ 30 min). Sections were examined on a JEOL1200 TEM (JEOL, Tokyo, Japan and JEOL USA, Peabody, MA, USA) at 60-80 kV and on an FEI Tecnai F20 TEM (Thermo Fisher Scientific) at 200 kV at Stanford Nano Shared Facilities, Palo Alto, CA, USA. Film (Eastman Kodak, Rochester, NY, USA and Fuji Photo Film) and digital (Olympus BX50 microscope) photography were used.

All animal and human tissue studies were performed after obtaining permissions from the appropriate committees and hospitals.

Results

Normal bone development

MOBLs synthesising woven bone and SOBLs synthesising lamellar bone

MOBLs synthesising woven bone and SOBLs synthesising lamellar bone on the woven bone scaffold are illustrated (Fig. 1,2). This terminology refers to normal, repair and pathological bone formation throughout all vertebrates. It encompasses initial woven and subsequent lamellar (parallel-fibred) bone formation leading to both eventual osteonal and fibrolamellar cortical tissue.

Woven bone formation closely followed by lamellar bone formation in normal development

Normal long bone formation in vertebrates followed a consistent pattern combining both intramembranous and endochondral mechanisms. Cortices were formed by the intramembranous mechanism mediated by the inner cambial layer of the periosteum, while metaphyseal and marrow trabeculae were formed by the endochondral mechanism mediated by the epiphyseal growth plates (physes), with bone deposited internally on a scaffold of calcified cartilage (Rivas and Shapiro, 2002). These two mechanisms also characterised axial bone development (vertebrae, ribs, pelvis).

Cortical and trabecular bone development: mouse, rabbit, pig; embryonic, postnatal

In the developing embryonic long bone, woven bone was formed initially by MOBLs of the inner (cambial) layer of the periosteum at the primary centre of ossification surrounding the mid-portion of each cartilage model. This represented the intramembranous mechanism of bone formation. Then, synthesis of lamellar bone occurred by SOBLs once a region of woven bone was sufficiently large to serve as a scaffold. Lamellar bone was invariably deposited on collections of woven bone synthesised by the periosteum. Then, woven bone synthesis passed towards both ends of the long bone eventually surrounding the developing physis and becoming a component of the perichondrial ossification groove of Ranvier. The bone surrounding the physis, referred to as the bony bark of Lacroix, was initially woven in conformation (like the rest of the cortex). At the deepest part of the groove, the inner (cambial) layer of the periosteum is continuous, with a dense cellular accumulation, a collection of pre-osteoblasts that are in the process of becoming MOBLs (Shapiro *et al.*, 1977). Then, woven bone formed in the depths of the groove and the outer part of the inner periosteal layer while the inner part

of the periosteal layer was composed increasingly of lamellar bone synthesised by SOBLs on initially woven collections. Photomicrographs from the inner cambial layer of a new-born rabbit femur and the groove region of a foetal piglet (Fig. 1,2) demonstrated the sequence of bone formation from pre-osteoblast to woven bone to early lamellar bone. Bone formed through the endochondral mechanism was deposited internally at the metaphysis on calcified cartilage cores extending from the physis (Fig. 3a-c). During periods of extremely rapid growth, woven bone was deposited on the cartilage cores initially and, then, quickly covered by lamellar bone (Fig. 3c). Much of the bone was synthesised directly on the calcified cartilage and was parallel-fibred (lamellar) since the cores served as scaffold for the SOBLs (Fig. 3a,b). Both woven bone and cartilage cores were resorbed by osteoclasts once their scaffold function was completed (Fig. 3b,d). Photomicrographs of lamellar on woven bone synthesis in pigs were similar

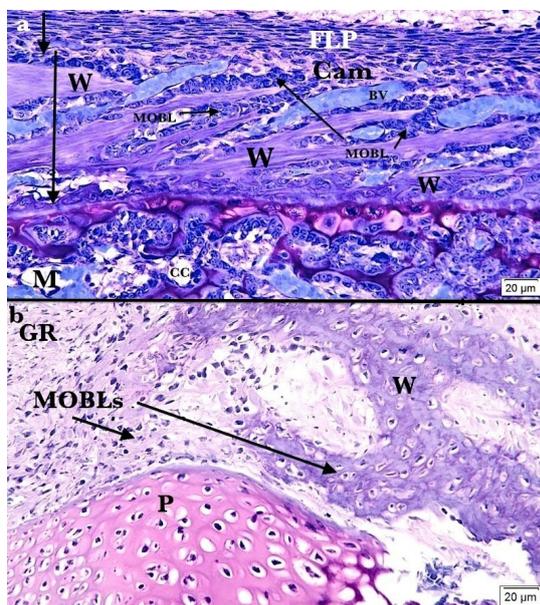


Fig. 1. Photomicrographs show regions of developing bone, periosteum and groove of Ranvier, where woven bone was prominent. (a) Photomicrograph shows a region of late embryonic rabbit femoral periosteum, cortex and adjacent metaphysis sectioned along the longitudinal axis. Short arrow at upper left corner outlines outer fibrous layer of periosteum; longer arrow below outlines inner cambial layer of periosteum and woven bone of cortex. W = woven bone; M = metaphysis; BV = blood vessel; CC = calcified cartilage within metaphysis; FLP = fibrous layer of periosteum; Cam = cambial layer of periosteum. Plastic-embedded section stained with 1 % toluidine blue. **(b)** Photomicrograph shows periphery of the physis (P) and adjacent perichondrial ossification groove of Ranvier (GR) in foetal piglet distal femur. On the right, woven bone (W) synthesis occurred at the depths of groove from MOBLs. This represents the furthest extension of periosteal intramembranous cortical bone formation. Plastic-embedded section stained with 1 % toluidine blue.

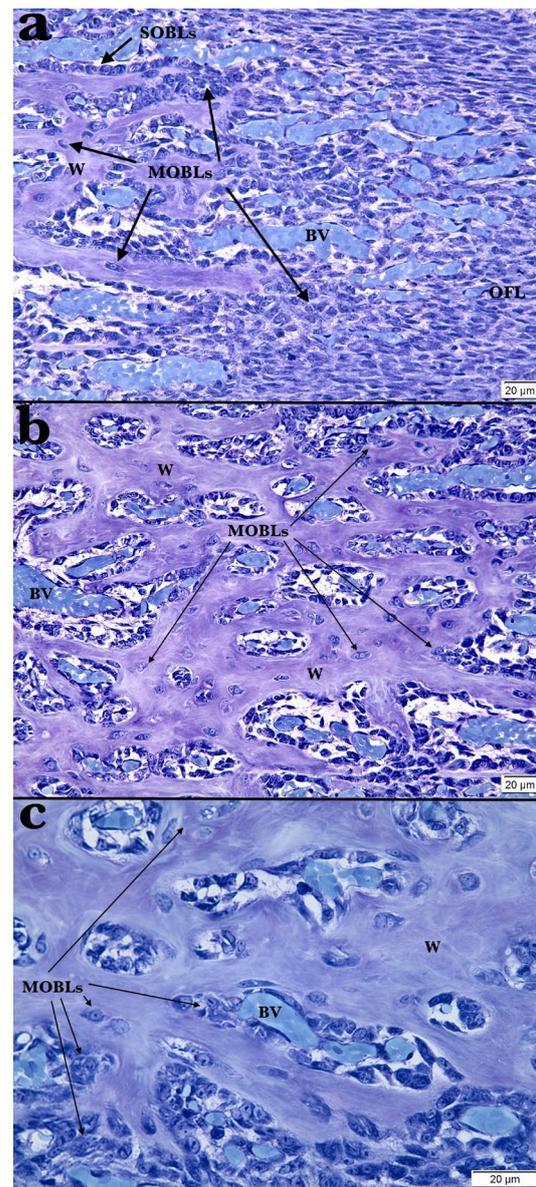


Fig. 2. Photomicrographs show developmental sequence in embryonic periosteum as MOBLs synthesised woven bone. (a) Oblique section through late embryonic rabbit femoral periosteum highlighted cambial layer with woven bone formation. OFL = outer fibrous layer (of periosteum); W = woven bone; BV = blood vessel (vessels are concentrated in the outer part of the inner cambial layer). SOBLs quickly forming on newly synthesised woven bone scaffolds. Plastic-embedded section stained with 1 % toluidine blue. **(b)** Oblique section through late embryonic rabbit femoral periosteum highlighted woven bone synthesis. Plastic-embedded section stained with 1 % toluidine blue. **(c)** Higher power photomicrograph of an oblique section through late embryonic rabbit femoral periosteum showed woven bone formation from mesenchymal osteoblasts throughout the section. Note close relationship of blood vessels with mesenchymal osteoblasts. Plastic-embedded section stained with 1 % toluidine blue.

throughout embryonic development and at 10 weeks post-natal (Fig. 4a-d, 5a,b). Piglet cortical bone was fibrolamellar, formed with repetitive layer-by-layer synthesis, with the woven bone scaffold maintained. The bone was reticular and plexiform rather than exclusively osteonal as in humans. Some primary osteons formed, but the repetitive fibrolamellar pattern was synthesised until skeletal maturation.

Human cortical and trabecular bone development (foetal, post-natal)

In 4-4 ½ month-old foetal long bones there was already extensive parallel-fibred lamellar bone deposited on woven bone initially synthesised by the periosteum and on calcified cartilage at the metaphyses (Fig. 5c, 6a-d). Examination by polarised-light microscopy highlighted this observation. By the new-born period, lamellar bone predominated over woven bone in the cortices. The pattern became exclusively lamellar in childhood, characterised by densely packed Haversian systems with interstitial lamellar bone interposed and no woven bone.

Repair bone models and mechanisms

Bone repaired itself by differing mechanisms (endochondral or intramembranous) depending

on the environment provided, such as length of gaps, stability between fragments and repair site vascularity.

Intramembranous bone repair in a stable mechanical environment; circular/cylindrical 2.4 mm cortical defect in rabbit femur diaphyses

As soon as a drilling defect through one cortex of the diaphysis of a young rabbit femur was done, blood filled the space and fibrinous arcades formed within the blood clot across the defect from wall to wall (Shapiro, 1988). Undifferentiated mesenchymal cells (narrow, elongated) lay parallel to the fibrinous arcade, in conjunction with vascularisation. They gradually assumed an oval to round shape as differentiation proceeded along the pre-osteoblast to MOBL line. The progressive developmental sequence from undifferentiated mesenchymal cells (Fig. 7a) to MOBLs, with increasing woven tissue synthesis (Fig. 7b), to SOBLs, synthesising lamellar on woven bone (Fig. 7c), was demonstrated. At 1 week, new bone formation beginning in the marrow advanced to the periphery of the defect (Fig. 7d).

Repair did not occur uniformly with the same tissue pattern across the entire defect. Initial new bone synthesis occurred peripherally apposed to the rim of

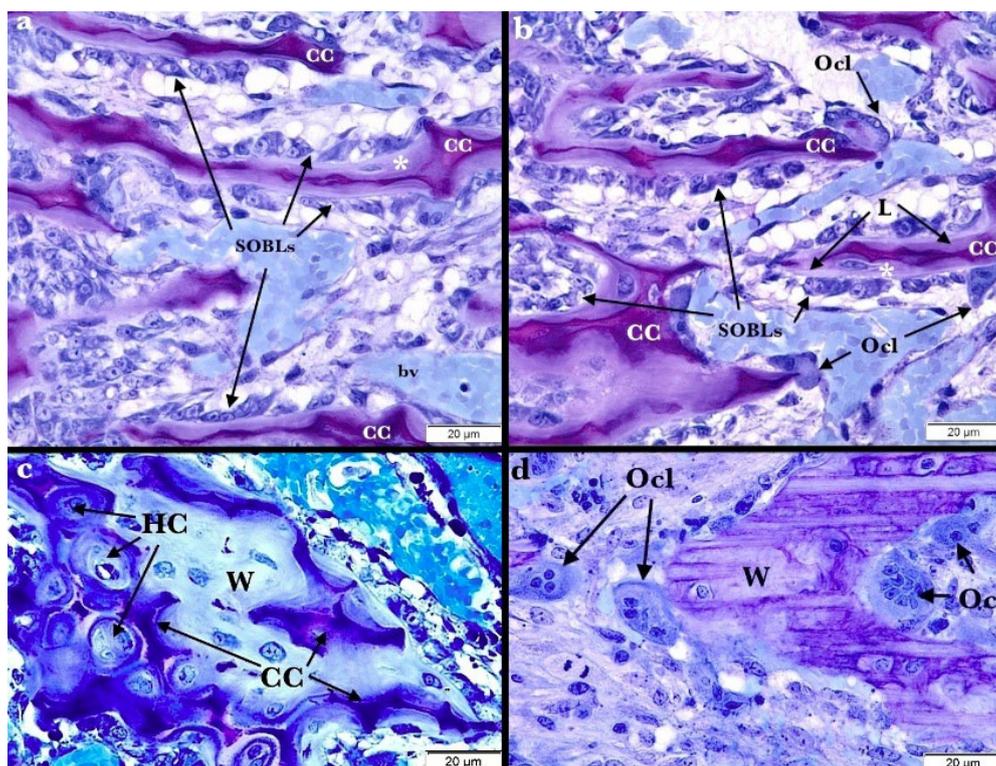


Fig. 3. Photomicrographs show examples of SOBLs synthesising lamellar bone on rigid calcified cartilage cores in metaphysis, woven bone synthesis adjacent to hypertrophic chondrocytes in metaphysis and osteoclasts resorbing woven bone. (a,b) In the metaphysis, lamellar bone was deposited directly onto the cartilage cores, which served as scaffolds. L = lamellar bone; * = lamellar bone with characteristic elongated osteocytes; CC = calcified cartilage cores; Ocl = osteoclasts; bv = blood vessel. (c) Woven bone (W) formation occurred immediately adjacent to lower physeal hypertrophic chondrocytes (HC) on and between calcified cartilage cores (CC). (d) Woven bone (W) at distraction osteogenesis site was undergoing resorption by multinucleated osteoclasts (Ocl). (a-c) From neonatal mouse distal femoral metaphysis. (d) From site of distraction osteogenesis.

lamellar cortical bone, with the central defect region healing last. The initial deposits on the cortex were woven but occasionally the persisting cortex serving as a scaffold allowed for a thin incomplete rim of direct lamellar bone synthesis. Examining an entire repair field from cortex to cortex at 2 weeks, adjacent to the periphery, woven matrix (initially synthesised) had become covered by a single-cell layer of SOBLs that, then, synthesised parallel-fibred lamellae (Fig. 7e). Advancing towards the centre, mesenchymal cells had differentiated into MOBLS surrounding themselves with a woven bone matrix. The central part of the defect was filled with undifferentiated mesenchymal cells. By 4 weeks, repair bone had filled the defect. Accumulations of woven bone were rimmed completely by SOBLs and lamellar bone and resorption of woven-bone segments by osteoclasts were underway. The proportion of lamellar bone increased quickly and, eventually, no woven bone persisted. The lamellar repair bone had become organised into Haversian system osteons. Polarised-light microscopy showed lamellar repair but with

fibril plane orientation perpendicular to the long axis of the persisting cortex even several weeks after injury.

Intramembranous bone repair in a stable mechanical environment; oval cortical defect 10 × 3 mm in chicken humerus
An oval cortical defect in the chicken also allowed for bone formation in a stable environment by the intramembranous mechanism, eliminating the need for healing through endochondral repair (Glimcher *et al.*, 1980). Bone tissue deposition was initiated against the rim of persisting cortical bone and, then, passed inwards towards the centre of the defect. The 4-week specimen was informative regarding matrix deposition. Bone at the periphery was already composed of osteonal lamellar bone synthesised on the initial woven bone deposits. Closer to the centre of the defect, woven bone predominated, with some SOBLs seen, while, in the centre of the defect, undifferentiated mesenchymal cells were differentiating into MOBLS and beginning to synthesise woven bone (Fig. 8a,b).

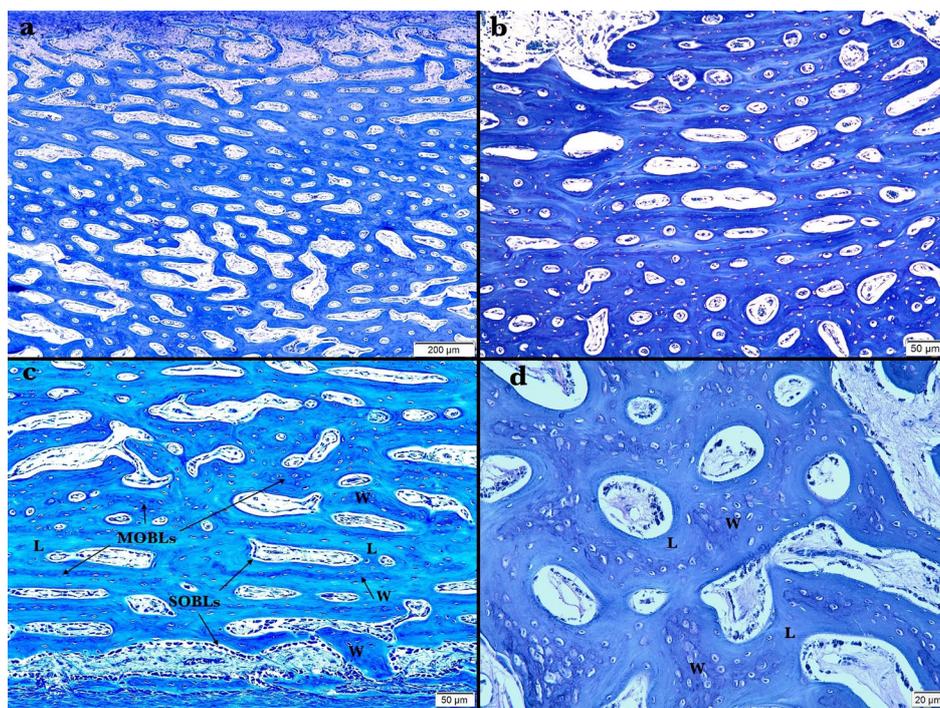


Fig. 4. Photomicrographs show late stage foetal piglet femoral cortices sectioned in the longitudinal plane. (a-d) All sections were plastic-embedded and stained with 1 % toluidine blue. (a) Cortical bone showed a characteristic fibrolamellar pattern. The outer fibrous layer of periosteum is on the top, with a thin layer of woven bone immediately below it; most of the cortical tissue is fibrolamellar in plexiform arrangement. (b) Parallel-fibred lamellar bone appear lighter blue while central cores of woven/fibrous tissue are darker blue. (c) Periosteum is at the bottom of the image. Fibrolamellar bone is seen; the use of partially-polarised illumination shows lamellar bone (L) to be lighter blue and woven bone (W) darker blue. Partially-polarised illumination for microscopy highlighted the woven and lamellar matrix components while still allowing the cellularity to be seen. The woven regions were more cellular than the lamellar regions. The cells within the lamellae were flattened (elliptical) and parallel to the long axis of the lamella. Woven bone formation predominated at the periosteum (at bottom) but SOBLs were deposited on the woven cores once formed. (d) Late stage foetal piglet femoral cortex is shown in cross-section. The fibrolamellar cortical bone was forming, with central cores of initially synthesised woven bone (W) surrounded by parallel fibred lamellar bone (L) synthesised on the woven cores. The lamellar regions were organising into primary osteons.

Endochondral bone repair in an unstable mechanical environment; rabbit femur

Fractured rabbit femora treated with lower extremity casts providing only partial bone fragment immobilisation led to healing by the endochondral/external bone callus repair mechanism (Shapiro, 2008; Shapiro, 2016). Once a stabilising cartilage callus formed at the fracture site, there was gradual vascularisation of the cartilaginous tissue mass leading to woven bone formation on calcified cartilage followed by deposition of lamellar bone on the initial woven collections (Fig. 8c,d). Periosteum distant from the fracture site synthesised intramembranous bone directly in the woven to lamellar sequence.

Intramembranous bone repair in a stable mechanical environment with controlled longitudinal distraction; rabbit tibia

Distraction osteogenesis enabled for simultaneous bone formation and lengthening without an intermediate cartilage phase. Stability was provided by external fixation and longitudinal distraction separated the fragments (Shapiro *et al.*, 1992; Shapiro, 2016). Following application of a tibial distraction device and complete osteotomy, bleeding at the site and formation of a fibrinous clot occurred. Blood vessel and undifferentiated mesenchymal cell invasion followed. Mesenchymal cells, initially thin and elongated, became oval to round as differentiation progressed along the osteoblast line. Repair was not uniform along the distraction gap. It began with woven bone synthesis adjacent to the cut cortices and occurred last at the central gap region. As gradual lengthening occurred, a range of regenerate bone was seen histologically. MOBLs and woven bone deposition were aligned longitudinally

along the long axis by mechanical distraction. Shortly after woven bone synthesis, SOBLs aligned preferentially on the surface and synthesised lamellar bone. At 2 weeks, the woven repair bone adjacent to the cut cortices was surrounded completely by lamellar bone. Towards the centre of the gap, woven bone was relatively more abundant as compared to lamellar bone while, centrally, a few undifferentiated mesenchymal cells were present. As lengthening and osteogenesis proceeded, woven bone became completely covered by lamellar bone and, then, resorbed by osteoclasts (Fig. 9a-e).

Other experimental models showing invariable synthesis of woven bone repair followed by lamellar bone deposition are summarised in Table 6.

Bone formation in pathological disorders

Osteogenesis imperfecta

Histological assessments on multiple bone samples from 30 patients with osteogenesis imperfecta revealed the progression of woven to lamellar bone formation in the varying types (Fig. 10a,b; 11a-d). In the most severe lethal perinatal variants (Sillence II), woven bone only in small amounts was synthesised; in less severe progressively deforming variants (Sillence III, IV), lamellar bone was synthesised on woven cores; while in the mildest benign autosomal dominant form (Sillence I), lamellar bone predominated in varying patterns [Maguire *et al.* (2011) Osteogenesis imperfecta bone: correlation of histologic structure and radiographic appearance. American Academy of Orthopaedic Surgeons annual meeting, San Diego, CA, USA; Shapiro, 1985; Shapiro, 2016; Shapiro and Flynn (2005) Variable structure of osteogenesis imperfecta bone. *J Bone Miner Res* 20 Suppl 1: S175; Van Dijk and Sillence, 2014].

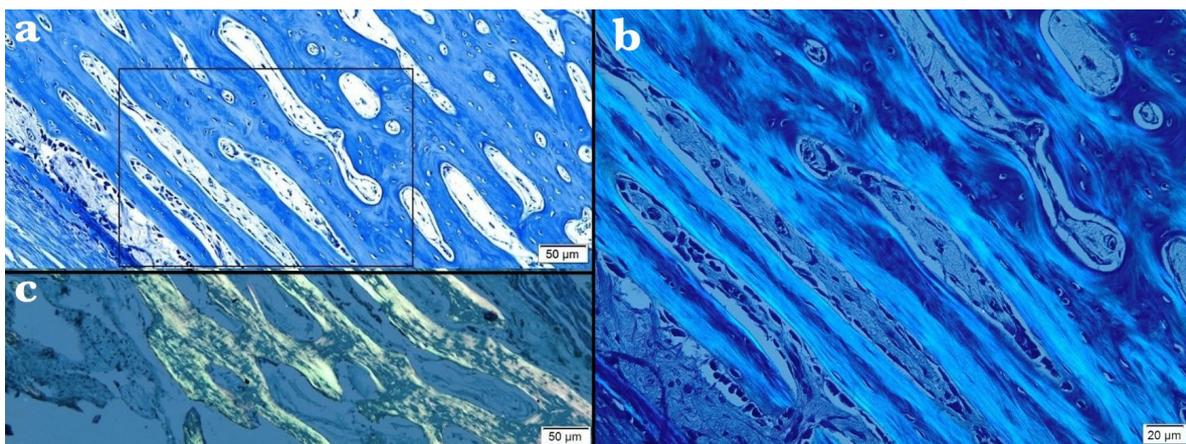


Fig. 5. Photomicrographs show matrix conformations in foetal pig and human bone with lamellar bone highlighted by polarised-light microscopy. (a,b) Photomicrographs of fibrolamellar bone from late stage foetal piglet femoral cortex are shown in longitudinal section. The same region is seen by (a) plain light microscopy (b) and polarised-light microscopy. (b) Higher magnification of the rectangle in a. Woven bone is in dark blue and lamellar bone in lighter blue. Plastic-embedded sections stained with 1 % toluidine blue. (c) Representative section of human foetal tibial cortex of a 4-4 ½ months old donor visualised by polarised-light microscopy shows a very high component of lamellar bone (yellow) that is as much as 50 % in some regions. The parallel fibres are stained in light yellow as compared with dark green staining of woven bone. Paraffin-wax-embedded section were stained with safranin O/fast green.

Osteosarcoma

The cell and matrix appearances of bone formed in highly malignant osteosarcoma lesions of the distal femur and proximal tibia were assessed in 10 adolescents (Shapiro, 1983; Shapiro and Eyre, 1982). Intra-osseous, transcortical and peri-osseous soft tissue foci in the malignant lesions elaborated a characteristic tumour osteoid matrix. Highly cellular woven bone formed from sufficiently differentiated tumour cells (Fig. 11e,f). Safranin O-fast green staining identified the matrix as woven bone (green stain), with no red staining that would indicate chondrocyte differentiation. Regardless of the amount of woven bone produced, there was no conformational switch to lamellar bone. Owing to rapid growth of these highly malignant lesions, woven bone was synthesised continuously by the tumour mass and often spread to surround necrotic pre-existing lamellar cortical bone and metaphyseal trabeculae. Osteosarcoma lesions as well as producing woven tumour bone (osteoid), also stimulated normal

reactive woven bone from the elevated periosteum at the periphery of the advancing tumour.

Lacunar-canalicular appearance in woven and lamellar bone

The lacunar-canalicular systems differed in appearance in woven and lamellar bone (Shapiro, 1988; Shapiro *et al.*, 1995; Shapiro, 1997; Shapiro, 2016). Cell shape (within the lacunae) and cell process pattern (within the canaliculi) reflected the orientation of the collagen matrices within which they lay (Fig. 12a-e). Normal cortical osteocytes in lamellar bone were elongated and elliptical in shape and lay parallel to the longitudinal orientation of the lamellae. Cell processes passed out from the osteocyte body at regular intervals from both ends of the cell and perpendicular to its walls at regular intervals. The regularity of the lacunar-canalicular pattern conformed to the organised lamellar collagen matrix. In contrast, woven bone osteoblasts and osteocytes were round to oval, had no specific orientation to

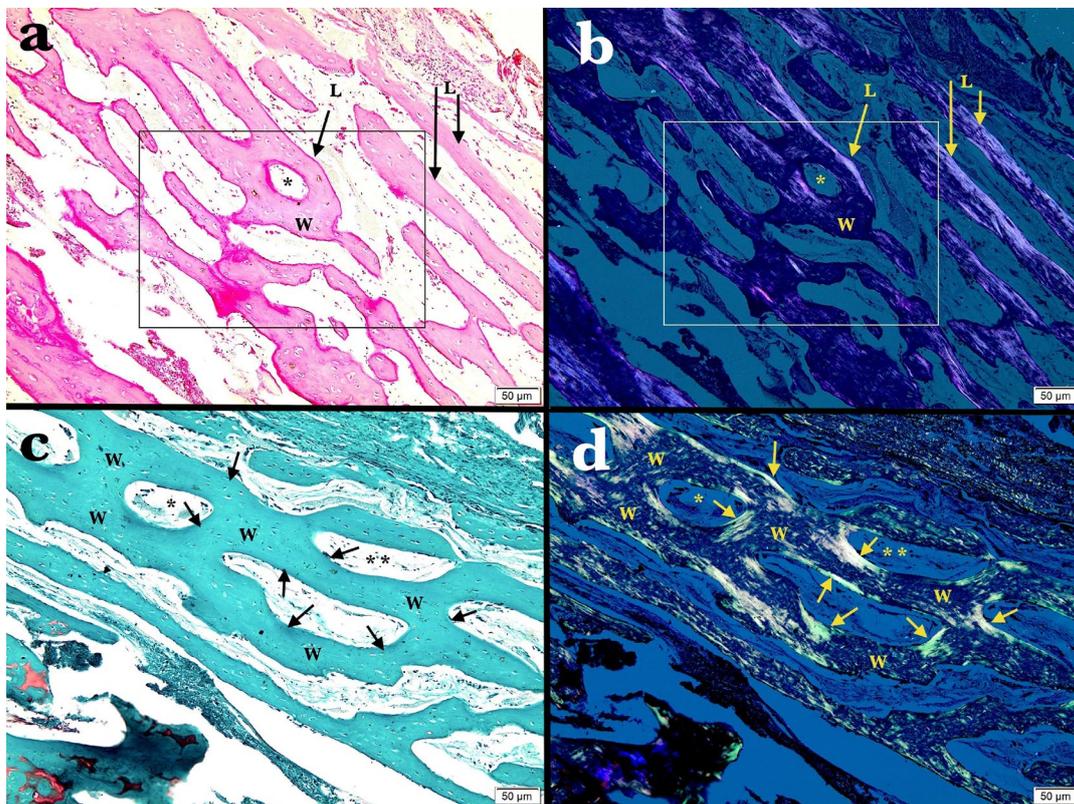


Fig. 6. Photomicrographs show paraffin-wax-embedded sections of human foetal 4-4 ½ months old tibial cortical bone. Sections were stained (a,b) with haematoxylin and eosin and (c,d) safranin O/fast green. Rectangles outline the same region in a and b. (a) While cellularity of the bone was evident, woven bone (W) and lamellar bone (L) were not clearly differentiated in the haematoxylin and eosin-stained section. * = same area in a and b. (b) A polarised-light microscopy image of the region in human foetal tibia outlined in a. Woven bone (W) is dark and lamellar bone (L) (arrows) light purple due to parallel-fibred tissue. Human foetal tibial cortical bone presented significant amounts of parallel-fibred lamellar components at 4-4 ½ months of intrauterine development. Lamellar bone regions represented as much as 25-33 % of cortical bone at this time. (c,d) Same section shown. W = woven bone; L = lamellar bone; * and ** = same areas. (c) Plain microscopy. Cortical bone is the central oblique strip of tissue; metaphyseal tissue and marrow are seen at lower left (red = calcified cartilage) and fibrous tissue overlying the bone is seen at upper right. (d) Polarised-light microscopy. Arrows point to parallel-fibred lamellar tissue (yellow). Polarised light highlighted the significant amount of parallel-fibred lamellar bone (yellow, arrows) already present in young foetal tissue.

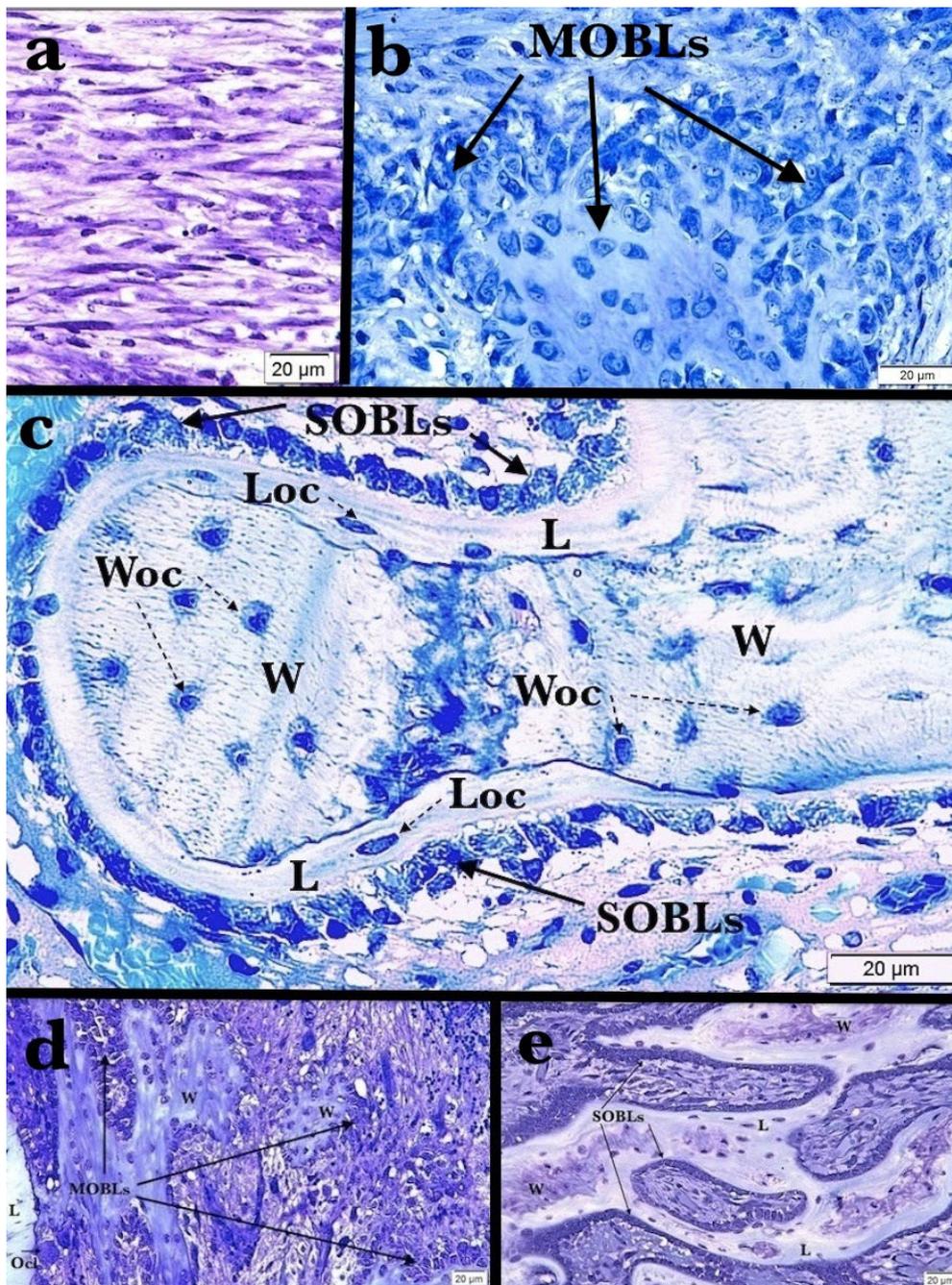


Fig. 7. Photomicrographs show range of cell and matrix appearances in bone repair. (a,e) Photomicrographs showing rabbit bone repair at different time periods after a drilling defect was made. (a,d,e) From a 2.4 mm unicortical femoral diaphyseal defect. (b,c) From the distraction osteogenesis model. All sections were plastic-embedded and stained with 1 % toluidine blue. (a) Undifferentiated mesenchymal cells, as seen in regions undergoing early repair 1 week post surgery, were flattened and elongated with a prominent nucleus. (b) Repair tissue from the distraction osteogenesis model in a rabbit tibia at 1 week. Undifferentiated cells are in the upper left corner, with progression to MOBLS seen. Centrally, MOBLS are surrounded by minimal amounts of matrix (right arrow) but, as seen in the lower half, increasing amounts of matrix were synthesised (left arrow). (c) Histology section from bone formation site within the elongating gap in distraction osteogenesis shows a woven bone core with lamellar bone synthesis on it. W = woven bone; Woc = woven bone osteocytes; L = lamellar bone; Loc = lamellar bone osteocytes; Ocl = osteoclast. (d) At 2 weeks, the developing sequence of repair is seen in a 2.4 mm defect going from the central region (on the right) to persisting cortex (on the left). Differentiation developed from undifferentiated mesenchymal cells, to preosteoblasts, to osteoblasts surrounding themselves with woven bone. W = woven bone; L = lamellar bone (in persisting cortex adjacent to the defect site); Ocl = osteoclast. (e) Woven (W) and lamellar (L) bone at periphery of repair site in a rabbit 2.4 mm unicortical femoral diaphyseal defect at 4 weeks.

immediately adjacent cells and their cell processes passed in irregular and random fashion through the woven matrix.

Polarised-light microscopy

Polarised-light microscopy provided a much more clear-cut definition of matrix conformation as shown in photomicrographs illustrating developing bone (Fig. 5b,c; 6b,d), fibrolamellar bone (Fig. 5b), repair bone (Fig. 8b,d) and pathological bone (Fig. 10b, 11d). Even in foetal human cortical bone, as early as 4-4½ months, there was relatively abundant parallel-fibred lamellar bone on woven bone cores (Fig. 5c; 6b,d). Observation by polarised-light microscopy confirmed the abrupt change between woven and lamellar conformations with no transition zone between the two types.

Histomorphometry

Cell area/total area ratios of osteoblasts/osteocytes in woven and lamellar bone

Examples of the quantitation process in the various types of bone conformation are shown in Fig. 12f for

woven bone, Fig. 12g for both woven and lamellar bone and Fig. 12h for lamellar bone. The cell area/total area index indicated the amount of matrix synthesised per cell. The higher the index, the greater the cellularity and the less the amount of matrix present. Samples assessed were normal human woven bone from foetal and new-born specimens, normal human lamellar bone from children and adult specimens and woven and lamellar bone specimens from patients with osteogenesis imperfecta. Normal woven bone from two foetal and two new-born individuals registered an index of 0.39 ± 0.012 (54 histological sections, 6,525 cells). Normal lamellar bone from 5 individuals, 5-40 years old, registered an index of 0.012 ± 0.005 (164 histological sections, 12,057 cells). In osteogenesis imperfecta, woven bone from 11 patients registered an index of 0.063 ± 0.03 (104 histological sections, 11,694 cells) and lamellar bone from 10 patients registered an index of 0.024 ± 0.009 (123 histological sections, 12,351 cells). Osteogenesis imperfecta bone was more cellular (*i.e.* synthesised less matrix per cell) than normal bone in both its woven and lamellar conformations

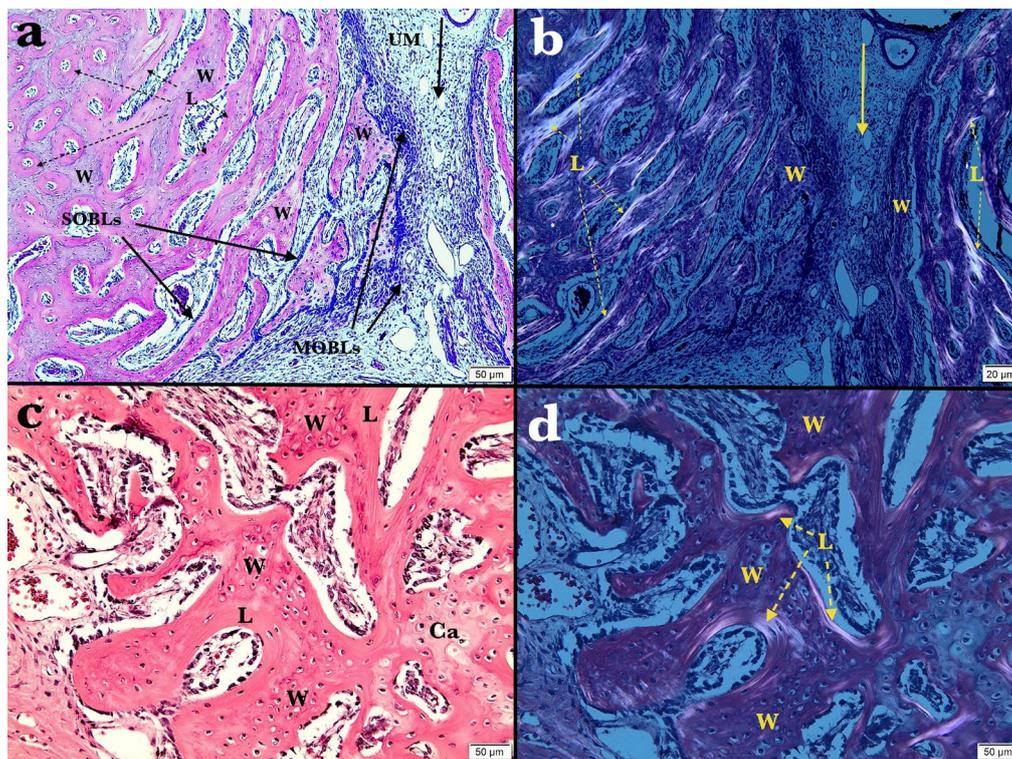


Fig. 8. Photomicrographs show bone repair in chicken and rabbit models. (a,b) Photomicrographs illustrating repair at a skeletally mature chicken humerus unicortical 3×10 mm defect at 4 weeks. Paraffin-wax-embedded sections stained with haematoxylin and eosin. Initial repair bone was at the periphery adjacent to the persisting cortex, with the central region last to repair. Solid vertical arrow at upper right = central part of defect; W = woven bone; L = lamellar bone; interrupted arrows = lamellar bone. (a) Repair was seen at 4 weeks post defect creation from the centre of the gap (on the right) to a region adjacent to the persisting cortex (on the far left). (b) Repair site illustrated with polarised-light imaging. Parallel-fibred lamellar bone appears bright yellow, while woven bone is dark. (c,d). Bone repair at site of endochondral callus in a rabbit femur treated with lower extremity cast immobilisation. Paraffin-wax-embedded sections stained with haematoxylin and eosin. (c) Repair was seen at 3 weeks. Ca = cartilage component of callus; W = woven bone; L = lamellar bone. (d) Polarised-light microscopy of section in c. Lamellar bone (L) is seen as bright staining and woven bone (W) remains dark. Ca = cartilage component of callus; interrupted arrows = lamellar bone.

but lamellar osteogenesis imperfecta bone was nevertheless less cellular than woven osteogenesis imperfecta bone.

Length/width ratios in woven and lamellar bone osteoblast/osteocytes

The ratio of length to width in cortical lamellar osteocytes is 3.5 : 1. In woven bone, osteoblasts/osteocytes recently differentiated from mesenchymal cells ratio is 1.8 : 1, consistent with their oval appearance. Once repair bone is switching to the lamellar orientation, the cells progressively change shape towards that of normal cortical osteocytes and at 4 weeks the ratio is 2.4 : 1 (Shapiro, 1988).

TEM

Woven and lamellar bone conformations were illustrated by electron microscopy in normal mouse cortical bone (Fig. 13a,b; 14a,b) and osteogenesis

imperfecta bone (Fig. 14c,d). The close juxtaposition of woven and lamellar matrices was demonstrated even at high levels of resolution, with relatively abrupt transition between the two conformations (Fig. 13b). In a segment shown to be lamellar by light and polarised-light microscopy, many of the collagen fibres were oriented parallel to the long axis of the bone but others were cut in cross-section indicating an orthogonal array (Fig. 14b). The developing orthogonal array was also seen in electron micrographs from osteogenesis imperfecta bone where active surface osteoblasts synthesised collagen.

Discussion

Biological significance of woven bone formation

The ability of mesenchymal osteoblasts to form woven bone *de novo*, at sites where no bone previously

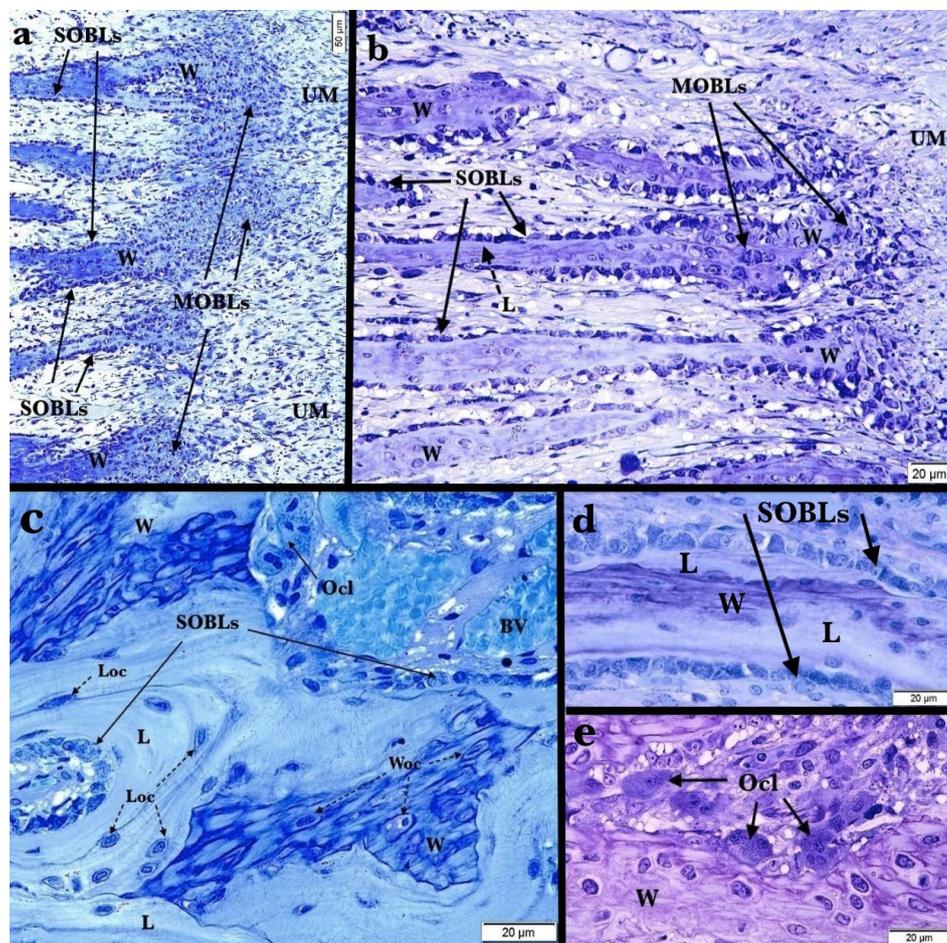


Fig. 9. Multiple photomicrographs are shown from a distraction osteogenesis model in a 3-month old rabbit tibia. All sections were plastic-embedded and stained with 1 % toluidine blue. (a) Woven bone (W) formation began at the central gap region on the right and progressively increased towards one of the cut cortices (not shown) on the left. Early repair at 2 weeks at the central gap region showed tissue differentiation from undifferentiated mesenchymal cells (UM) to woven bone. (b) Higher power view closer towards one of the cut bone ends shows mesenchymal osteoblasts well and elongated woven bone (W) collections. UM = undifferentiated mesenchymal cells. (c) Lamellar bone (L) on woven bone (W) is illustrated at 4 weeks. Woc = woven bone osteocyte; Loc = lamellar bone osteocyte; Ocl = osteoclast; BV = blood vessel. Plastic-embedded sections stained with 1 % toluidine blue. (d) Lengthened tissue from 4 weeks specimen. W = woven bone; L = lamellar bone. (e) Once woven bone (W) served as a scaffold it was resorbed by multinucleated osteoclasts (Ocl, arrows).

Table 6. Bone repair in additional experimental models inducing invariable woven to lamellar bone formation.

Bone repair following full-thickness articular cartilage defects into subchondral bone	Full-thickness 2.4 mm diameter articular cartilage defects were made in skeletally mature rabbit proximal tibiae through underlying subchondral bone into the marrow to assess articular cartilage repair. The defects induce subchondral bone repair below the articular cartilage and adjacent to the epiphyseal lamellar trabecular bone (Shapiro <i>et al.</i> , 1993). Undifferentiated mesenchymal cells synthesise woven bone adjacent to the subchondral bone plate and lamellar bone trabeculae. Bone repair continue in the subchondral region with SOBLs aligning on woven bone to synthesise lamellar bone.
Bone repair following transphyseal cartilage defects	Transphyseal defects through proximal tibial growth plate in immature rabbits elicit physeal repair responses, as observed by magnetic resonance imaging and histology (Jaramillo <i>et al.</i> , 1990). Vascularised tissue with undifferentiated mesenchymal cells from epiphyseal and metaphyseal sides migrate into the gap and MOBLs synthesise woven bone against the edges of the physeal cartilage. After several days, woven bone from both regions form a transphyseal bone bridge. Then, woven bone is covered by SOBLs to form a transphyseal lamellar bone bridge.
Bone repair following induction of ischemia (avascular necrosis): piglet femoral head	Avascular necrosis was induced in 3-week old piglet hips by intracapsular circumferential ligation at the base of the femoral neck (Shapiro <i>et al.</i> , 2009a). This caused complete femoral head ischemia followed by repair consistent with Legg-Calvé-Perthes disease. Histological studies at fragmentation stage showed vascularised mesenchymal tissue entering femoral head between articular and physeal cartilage. Mesenchymal tissues differentiate to MOBLs synthesising woven bone that is covered by SOBLs synthesising lamellar bone on woven scaffold.

existed, indicates a potent synthetic capability. Then, woven bone has a major influence on overall bone formation providing a scaffold or template on which stronger, more abundant lamellar bone is synthesised. The present study demonstrated the invariable presence of woven bone across the spectrum of vertebrate bone formation in development, repair and pathological bone disorders.

Bone has a well-ordered structural hierarchy with specific supportive functions (Lakes, 1993). Its three-dimensional structure, generally presented as the specific ordering of lamellar tissue, has been assessed in detail (Cohen and Harris, 1958; Gebhardt, 1906; Ghazanfari *et al.*, 2016; Marotti, 1993; Marotti *et al.*, 2013; Petersen, 1930; Reznikov *et al.*, 2014; Trelstad *et al.*, 1976; Wagermaier *et al.*, 2006; Weiner and Traub, 1992). Formation and maintenance of bone is due to a unique combination of cellular and molecular events interacting with biophysical influences (biophysical defined as the theories, laws and methods of physics applied to understanding biological phenomena).

The mesenchymal cell and its relationship to osteoblast and osteocyte formation

Maximow (1927) showed that undifferentiated mesenchymal cells serve as precursors for both the haematopoietic and connective tissues. He adopted the word “mesenchymal”, used originally to refer to the embryonic tissue layer, for post-natal tissue descriptions and used the term “stem cell” to refer to the functional potentialities of these undifferentiated cells. The shapes of mesenchymal cells change with development due to extrinsic passive deformation from their environment, such as the fibrinous reticulum of blood clots, and intrinsic transformation based on intracellular synthesis and formation of “major processes” or protrusions (Weiss and Garber, 1952). Weiss and Garber (1952) demonstrated changes in cell shape ranging from the initial narrow

elongated bipolar cells to oval or plump multipolar cells (polarity referring to the number and positioning of cell processes) as differentiation progresses. Fig. 7a shows early mesenchymal cell collections; at higher magnifications, changes to elliptical and oval shapes, with multiple cytoplasmic elongations, were seen as differentiation towards pre-osteoblasts occurred. The use of the term mesenchymal osteoblast derives from these considerations. Mesenchymal cell/stem cell function at the molecular level is being actively investigated (Kfoury and Scadden, 2015).

Differing osteoblast types synthesising woven and lamellar bone

Histological studies make it abundantly clear that initial bone matrix formation is made of woven bone, with a random array of collagen fibres synthesised across the spectrum of normal development, repair and pathological processes. This initial woven bone synthesis is performed by MOBLs. Once enough tissue has been synthesised, there is a marked change in bone cell and matrix deposition patterns as osteoblasts align in an orderly linear array on the surface of woven bone accumulations and deposit collagen matrix in an organised parallel-fibred lamellar pattern. These osteoblasts are referred to as SOBLs (Shapiro, 1988; Shapiro, 2008; Shapiro, 2016). While mesenchymal osteoblasts form woven bone where no bone previously existed, subsequent lamellar bone is formed by surface osteoblasts where there is a scaffold or template along which lamellar bone can be positioned. Most commonly, woven bone forms this scaffold, although lamellar bone can be deposited initially where other tissues provide the scaffold (Table 7).

The terms MOBLs, forming woven bone, and SOBLs, forming lamellar bone, indicate differing osteoblast functions. It is not known whether these two now clearly defined osteoblast types represent

distinct genetic lineages or whether they stem from a single osteoblast lineage that responds in different ways to differing biophysical stimuli. There is considerable mobility of many cell precursors, including undifferentiated and early differentiating mesenchymal cells, in embryonic, developmental and repair states as they migrate to position themselves for specific roles. MOBLs actively migrate to and further differentiate at their functional sites – since they are not initially present in the limb bud and cartilage model stages of development, at repair sites immediately at time of fracture or osteotomy – and at sites of early pathological bone formation. In addition, MOBLs synthesise woven bone rapidly and *de novo* at sites where no bone is present. These actions are the opposite of static and are, in effect, more dynamic than the slower oriented actions of SOBLs, synthesising lamellar bone on pre-existing surfaces. SOBLs are present in the undifferentiated mesenchymal cell pool adjacent to sites of woven bone formation and simply align themselves on the surface of a woven bone scaffold once it is present

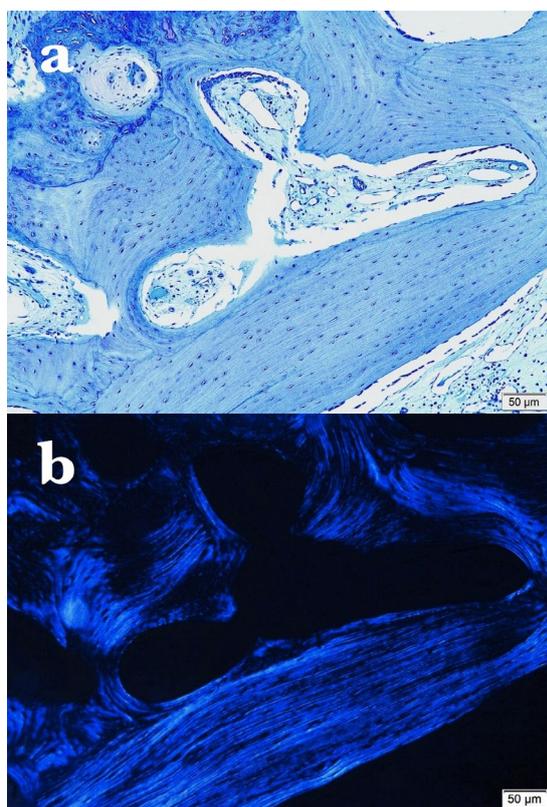


Fig. 10. Photomicrographs of osteogenesis imperfecta bone are shown. (a) Mosaic pattern of lamellar bone from tibia was evident in photomicrograph from patient with type III progressively deforming osteogenesis imperfecta, at the milder end of the spectrum. Most of the tissue was lamellar in organisation but a few areas of woven bone persisted. Plastic-embedded section stained with 1 % toluidine blue. (b) Polarised-light microscopy image of the same tissue section as shown in **a** demonstrating the lamellar structure of bone, with parallel-fibred tissue staining bright purple.

to a sufficient extent. Amprino (1947) observed that woven bone is synthesised faster than lamellar bone. This concept has been widely accepted and more recently quantified (de Margerie *et al.*, 2002). The descriptive MOBL/SOBL terminology (compared with the static osteogenesis/dynamic osteogenesis terminology) is more consistent with understanding woven bone/lamellar bone synthesis across the entire spectrum of vertebrate bone formation. Familiarity with each set of terms is important since both refer to the concept of differing functions for the two types of osteoblasts.

Bone matrix self-assembly

Bone tissue conformation, with its repetitive pattern of woven and lamellar formation, is due to obligate self-assembly of collagen molecules in the extra-cellular space. Self-assembly, the formation of supramolecular structures by physico-chemical phenomena alone (without cellular involvement) is characterised by the spontaneous organisation of molecules into stable aggregates through non-covalent bonds under equilibrium conditions (Boncheva and Whitesides, 2005; Whitesides and Boncheva, 2002). Self-assembly is a well-defined concept (Halpern, 2002; Lehn, 2002), has been documented in many biological systems (Bard, 1992) and is particularly referable to collagen formation (Fang *et al.*, 2013; Kadler *et al.*, 1996; Mosser *et al.*, 2006; O'Leary *et al.*, 2011; Silver *et al.*, 2003; Zhu and Kaufman, 2014). Collagen fibrils have been shown to aggregate *in vitro* in cell-free systems (Bouligand *et al.*, 1985). Orthogonal arrays of collagen in lamellar bone, seen by polarised-light and electron microscopy, are additional examples of self-assembly mechanisms of matrix conformation reflecting the biophysical search for a stable conformation. In normal bone, this pattern has specific temporal and spatial features; in repair bone, the specific features are also present but are modified by the biophysical environment within which repair occurs. In pathological bone, the same specific features are also present with modifications dependent on how the specific disorder affects the amount of bone formation.

Palaeohistology

Woven bone deposition prior to parallel-fibred and lamellar bone deposition also appears to be invariable in fossil bones from multiple extinct vertebrate species, based on palaeohistology studies. For purposes of the present overview of woven bone, the most important palaeohistology contribution is the recognition of invariable woven bone formation preceding lamellar bone formation. In many mammals as well as reptiles, amphibians and birds woven bone is not only prominent in embryonic cortical bone but persists and is repetitively synthesised postnatally until skeletal maturity (Barrera *et al.*, 2016; de Ricqlès, 1975; de Ricqlès, 1976; Francillon-Vieillot *et al.*, 1990; Förster and Goldbach, 1954; Gross, 1934; Locke, 2004; Magal *et al.*, 2014; Marín-Moratalla *et al.*, 2013;

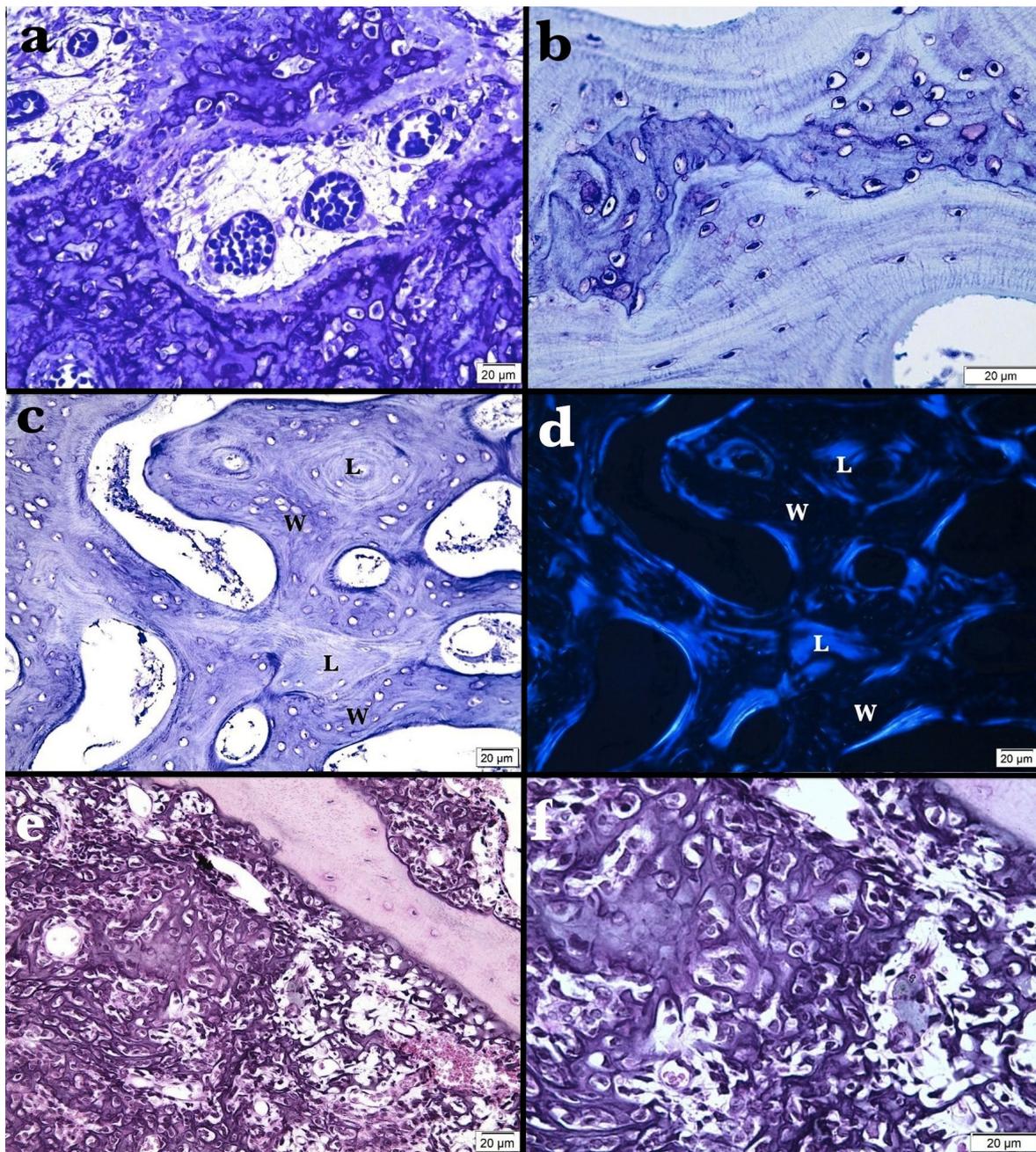


Fig. 11. Photomicrographs show osteogenesis imperfecta and osteosarcoma bone tissue. (a-d) Photomicrographs showing osteogenesis imperfecta tissue. All sections were plastic-embedded and stained with 1 % toluidine blue. (a) Formation of hypercellular woven bone characterised femoral cortical bone in severely affected lethal perinatal type II osteogenesis imperfecta patient. Note the prominent vascularity (at top). (b) High power photomicrograph from a segment of deformed tibia in another patient with type III osteogenesis imperfecta showing characteristic central woven bone tissue (synthesised initially) surrounded by bone in lamellar conformation. Note the elliptical shape of the lamellar bone lacunae (osteocytes) and the regular pattern of orientation of the numerous lamellar canaliculi. (c) Lamellar bone (L) formation on woven cores (W) shown in another patient with progressively deforming type III osteogenesis imperfecta. Note differences in cellularity between the two types. (d) The same section as shown in a assessed by polarised-light microscopy; lamellar bone (L) is bright purple, with woven bone (W) collections in dark purple. (e) Tumour osteoid from a distal femoral adolescent osteosarcoma was deposited in a woven conformation. The tumour osteoid surrounded a pre-existing lamellar trabeculum, with no surface osteoblast differentiation. (f) Osteosarcoma tissue at higher magnification showing the woven nature of the osteoid. (e,f) Paraffin-wax embedded and stained with haematoxylin and eosin.

Marín-Moratalla *et al.*, 2014; Montoya-Sanhueza and Chinsamy, 2017; Prondvai *et al.*, 2014; Ray *et al.*, 2005; Stein and Prondvai, 2014).

Gross (1934) referred to fibre bone (“faserknochen”) in many vertebrate species, while describing early woven and lamellar bone. de Ricqlès (1975) defined patterns of fibrolamellar, lamellar and Haversian bone that have become the standard for palaeohistology studies. Stein and Prondvai (2014) reinterpreted cortical bone structure based on studies using multi-planar sectioning and polarised-light microscopy. They felt that woven bone was more limited in amount than previously described in bone defined as fibrolamellar. They considered cortical bone tissue to be composed almost exclusively of highly organised primary bone (HOPB) consisting of both lamellar and non-lamellar components. The non-lamellar components (effectively non-osteonal) were considered to be parallel-fibred collagenous tissue rather than woven bone, based on anisotropic polarisation in differing planes and the presence of elongated elliptical-shaped lacunae characteristic of lamellar bone. Their interpretation used MOBL/SOBL or static/dynamic osteogenesis developmental considerations, limiting woven bone designations to MOBL/static-osteogenesis-derived tissue, with all HOPB bone representing SOBL/dynamic-osteogenesis-derived tissue. Then, a combined terminology was proposed defining a woven-parallel complex to recognise developmental aspects and retaining a more limited role for fibrolamellar bone (Prondvai *et al.*, 2014). Palaeohistology definitions of cortical bone structure are summarised in Table 2 and 3a,b.

Woven bone in relation to bone repair models

Cortical defect models

Unicortical diaphyseal rabbit and chicken models permit bone repair in a stable mechanical environment. In these models, a variable wave of tissue differentiation formed from cortex to cortex. At 3–4 weeks, histological sections showed an occasional incomplete thin rim of lamellar bone (synthesised initially on the persisting cortical scaffold), lamellar tissue osteons predominating at the site of earliest woven bone repair at the periphery of the defect (having undergone the lamellar on woven progression), collections of woven bone closer to the centre of the defect on which parallel-fibred lamellae were being deposited by SOBLs, woven bone alone synthesised by MOBLs even closer to the centre and, at the centre of the defect, undifferentiated mesenchymal cells persisted, while beginning differentiating into MOBLs. The reverse sequence was seen from the centre to the opposite cortex.

Many studies have defined the histological profile of bone formation in stable experimental environments using either plate compression of osteotomies or variable-diameter unicortical defects (Müller, 1978; Perren *et al.*, 1969; Perren, 1979; Schenk and Willenegger, 1963; Schenk and Willenegger,

1964; Schenk and Willenegger, 1967; Schenk and Willenegger, 1977). These studies have shown how sensitive cells and matrices are to relatively subtle changes in their biophysical environment. Primary bone repair or contact healing refers to the immediate formation of lamellar repair bone by SOBLs from adjacent Haversian systems parallel to the long axis of the bone where the fragments are reduced anatomically and stabilised rigidly eliminating any interfragmentary gap. Lamellar bone repair crosses the osteotomy line without a cartilage or woven bone phase. Direct transformational bone repair or gap healing occurs in a stable environment, without a cartilage phase, where there is an interfragmentary gap of 0.2 mm or larger, allowing woven bone formation initially followed by lamellar bone at right angles to the longitudinal axis along the axis of vascular and mesenchymal cell invasion. Then, the lamellar bone undergoes remodelling (transformational component) to lie parallel to the Haversian systems of the long axis. The relationship of gap extent and tissue conformation is detailed in Table 7.

Distraction osteogenesis

Bone deposition in distraction osteogenesis also began adjacent to each cortex, with persistence of undifferentiated mesenchymal cells in the central gap between bone ends. Woven bone initiated repair, was elongated along the longitudinal axis with distraction and served as a scaffold for SOBLs forming trabecular and cortical bone (Fig. 9a–e) (Schuelke *et al.*, 2018).

Additional examples of invariable woven bone formation in experimental situations

Other examples of invariable woven bone formation preceding lamellar bone formation are shown, including endochondral callus bone repair and others, as summarised in Table 6.

Pathological bone formation

Osteogenesis imperfecta

Woven/lamellar bone conformation also predominates in pathological bone formation as seen particularly well in osteogenesis imperfecta. The relative woven/lamellar bone relationship defines the clinical severity of the disorder (Falvo and Bullough, 1973; Jaffe, 1972; Shapiro, 2016; Weber, 1930). In the lethal perinatal Sillence II variant, woven bone alone was synthesised, although in such limited amounts that parallel-fibred lamellar bone was rarely seen (Fig. 11a). In severe progressively deforming variants (Sillence III), histology showed combinations of woven and lamellar bone (Fig. 11b–d). Once enough collagen has been synthesised as woven bone, subsequent new bone deposited on the woven scaffold is lamellar. The milder the involvement is, the more lamellar bone is synthesised, but woven bone cores tend to persist throughout childhood. Haversian system osteonal formation proceeds but the osteons are rarely fully compacted and the cortex is thinner than

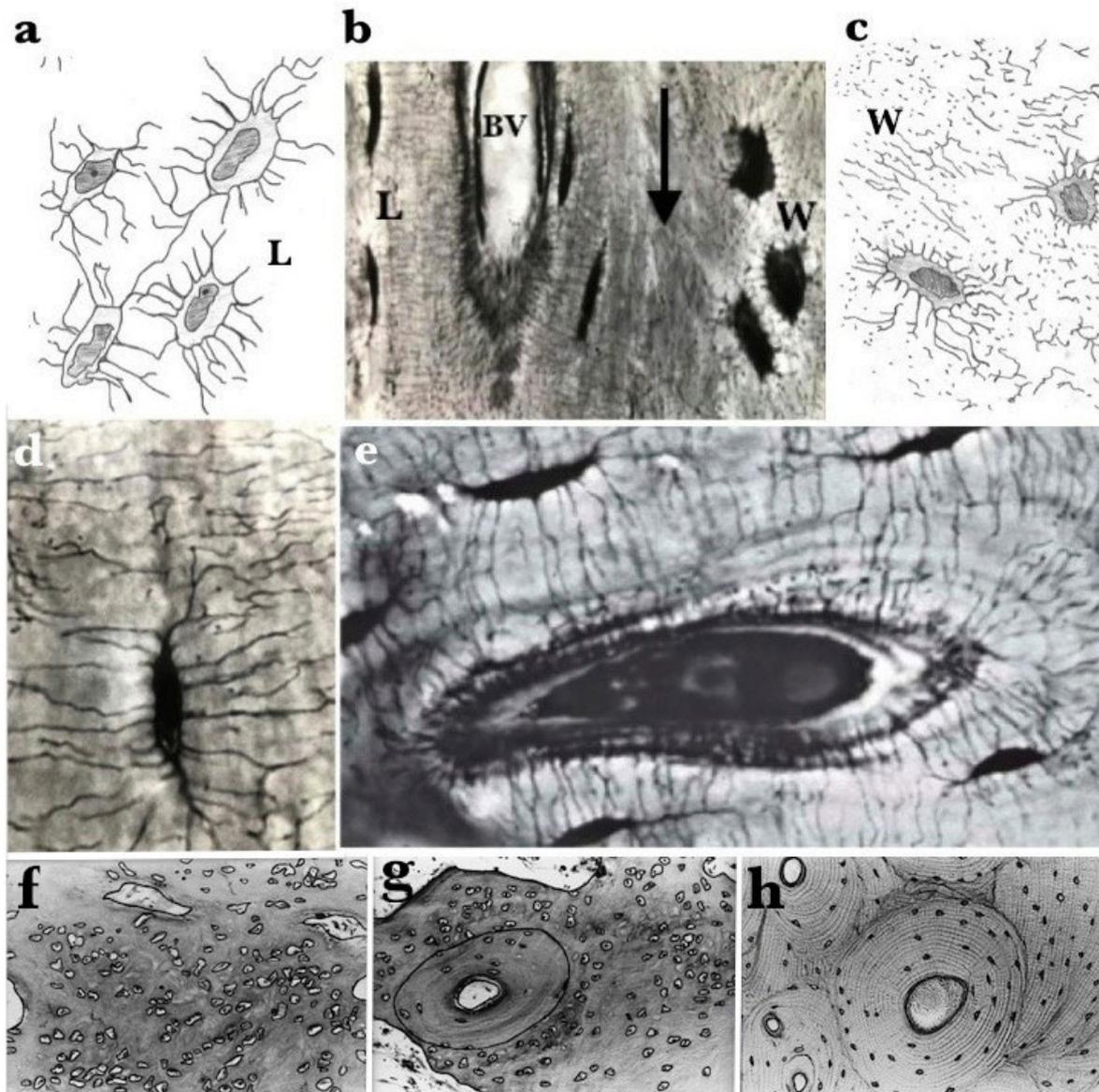


Fig. 12. Bone lacunae and canaliculi in differing matrix conformations are shown. (a) Tracings from photomicrographs of rabbit 1 week old femoral cortical bone outlining the lacunar-canalicular system in active-forming lamellar bone (L). Osteocytes were elongating and the canaliculi tended to radiate at right angles from the cell walls (interrupted arrows). A canaliculus at both ends of an osteocyte united one cell with an adjacent cell along the same long axis (solid arrow). (b) Photomicrograph of heavily stained 1 week old rabbit cortical bone showing, on the left, lamellar bone (L) around a blood vessel (BV), with flattened elongated osteocytes, with characteristic organised canaliculi parallel to and at right angles to the lengthened osteocyte lacunae reflecting their passage through the parallel-fibred lamellar matrices. On the right, woven bone (W) osteoblasts, with randomly arrayed canaliculi. Arrow = demarcation of lamellar and woven bone. (c) Tracings of round to oval woven bone (W) cells showing canaliculi randomly oriented reflecting their passage through the woven matrices. (d) A heavily stained single osteocyte with cell processes (lacunar/canalicular system) in lamellar bone showing the elliptical, elongated cell shape and the well-oriented canaliculi traveling within the ordered lamellar matrix. (e) Oblique section in rabbit lamellar bone showing elongated osteocytes in lamellae and their organised canaliculi linking osteocytes to lining surface osteoblasts adjacent to a central blood vessel. (b,d,e) From plastic-embedded sections stained with 1 % toluidine blue. Histomorphometry at light microscopy level characterising woven and lamellar bone regarding cell area/matrix area ratios. In these examples, each cell perimeter is outlined in black. Quantitation is done on the digitising app of the microscope. (f,g,h) From osteogenesis imperfecta bone. Section of (f) woven bone, (g) both woven and lamellar bone and (h) lamellar bone with osteons cut in the cross-sectional plane. The differences in cellularity were evident by simple observation but quantitation allowed for more definitive comparisons.

normal. When assessing bone structure by histology in mild autosomal dominant osteogenesis imperfecta (Sillence I), the tissue often cannot be differentiated from the normal one, showing extensive osteonal lamellar bone and no traces of woven bone. In normal bone, cell area/total area ratios are markedly different between woven and lamellar bone, with woven bone always more cellular than lamellar bone, with less matrix per cell area. There is abrupt apposition of lamellar on woven bone with no intermediate forms seen. These observations in osteogenesis imperfecta bone support the contention that the structure of bone is an obligate phenomenon of supra-molecular organisation where self-assembly of matrices is based on biophysical rather than strictly molecular constraints. The mutations of collagen cause less matrix to be synthesised leading to persistence of immature matrix patterns.

Osteosarcoma

The earliest histopathology of osteogenic sarcoma (osteosarcoma) tissue showed what appeared to be woven bone, although it was generally referred to as tumour bone or tumour osteoid (Jaffe, 1956). Direct production of osteoid matrix by sarcomatous tumour

cells is required to classify a lesion as an osteosarcoma. The tumour osteoid has been increasingly referred to as woven bone in descriptions and photomicrographs of osteosarcoma lesions (Czerniak, 2016a; Mirra *et al.*, 1989). The characteristic finding with malignant neoplastic tumour bone (distinct from normal or repair bone) was the infiltration of highly cellular woven bone tissue into lamellar bone regions in uncontrolled fashion, with no tendency of SOBLs to synthesise new lamellar bone on the tumour woven bone (Fig. 11e,f). As tumour cells invade metaphyseal and cortical regions, woven tumour bone surrounds necrotic lamellar bone spicules. Mineralisation of the tumour osteoid produces radiographic sclerosis. Peripheral reactive periosteal bone in osteosarcoma lesions is woven before inducing an orderly sequence of SOBLs forming lamellar bone. Efforts are being made to determine if osteosarcoma cells are mesenchymal stem cells or osteoblast committed cells (Mutsaers and Walkley, 2014).

Other pathological bone disorders

Paget's disease of bone (osteitis deformans) occurs in adults, generally 50 years old or older. Histopathological descriptions have shown woven bone formation early in the active stages of the disorder, although eventually the predominant findings are osteoclastic bone resorption and synthesis of lamellar bone with a mosaic pattern (Dickson *et al.*, 1945; Freund, 1929; Jaffe, 1933; Knaggs, 1925; Meunier *et al.*, 1980; Schmorl, 1932; Seitz *et al.*, 2009). Highly cellular collections of woven bone are prominent in benign bone tumours and other pathological bone-forming lesions such as osteoid osteoma, within the cellular fibrovascular central nidus, osteoblastoma, where the slow growing disorder allows for formation of SOBLs and lamellar bone on woven deposits (Mirra *et al.*, 1989; Czerniak, 2016b), fibrous dysplasia (Czerniak, 2016c), secondary hyperparathyroidism (Krempien *et al.*, 1975) and chronic osteomyelitis (Mirra *et al.*, 1989).

Classification of the stages of woven bone formation

Based on the authors' studies reported in the present manuscript, four specific stages of woven bone formation were defined: stage I, early differentiation of pre-osteoblasts from undifferentiated mesenchymal cells; stage II, MOBLs surrounding themselves in a 360° arc with randomly oriented matrix fibres; stage III, woven matrix acting as a scaffold on which SOBLs begin to synthesise bone in parallel-fibred lamellar conformation; stage IV, progressive relative diminution of woven bone in the woven bone/lamellar bone complex. Stages II and IV are further subdivided (in a, b and c) by shifting cell area/matrix area and woven bone/lamellar bone relationships. To link woven bone formation to the entire sequence of bone tissue development, stage O and V are also defined. Stage O is characterised by mesenchymal cell development from undifferentiated thin, flattened

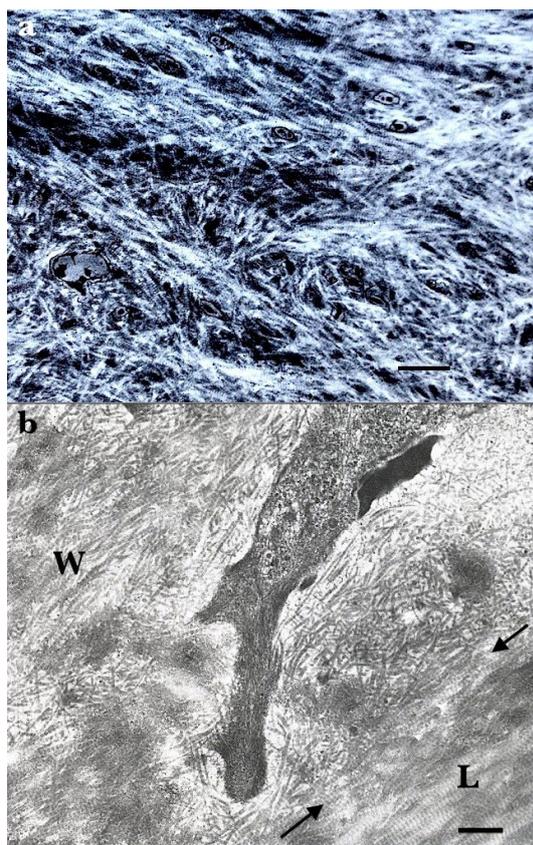


Fig. 13. Collagen appearances in woven and lamellar bone are shown by TEM. (a) Transmission electron micrograph in mouse neonatal femoral cortical bone illustrating woven bone. Collagen is seen with fibrils in random array. Scale bar: 667 nm. (b) Transmission electron micrograph in mouse neonatal femoral cortical bone showing the juxtaposition of lamellar (L) on woven (W) bone at lower right. Scale bar: 765 nm.

elongated cells to progressively differentiating oval to round cells destined to form an osteoblast line. Stage V is when all bone seen in a region is lamellar. The classification is presented in Table 7.

Woven and lamellar bone in relation to the two bone formation mechanisms

Completely formed cortical and trabecular bone are considered to be the same regarding molecular components and lamellar tissue structure, even though they develop following different intramembranous and endochondral bone mechanisms. Although the final bone produced is the same, the mechanisms differ early on regarding the scaffold tissue being replaced: woven bone at the cortices during intramembranous bone formation and calcified cartilage cores at internal marrow/trabecular sites during endochondral bone formation. The role of

woven bone formation, serving as a scaffold for lamellar bone deposition, is detailed in the present report but additional situations where a firm scaffold other than woven bone leads to direct lamellar bone formation were seen (Table 7; Fig. 3a-c). Woven bone was removed by osteoclasts once its role as a scaffold for lamellar bone deposition was completed. Calcified cartilage cores were also removed by osteoclasts in the process of transforming metaphyseal region trabeculae into lamellar bone (Fig. 3b,d; 9c,e).

Demonstration of woven and lamellar bone

Woven bone can be distinguished from lamellar bone (Table 4). Many of the distinctions are demonstrable by light microscopy: i) there is an abrupt transition from woven to lamellar bone, well-defined lamellar bone being deposited on collections of well-defined woven bone without the interposition of a transitional

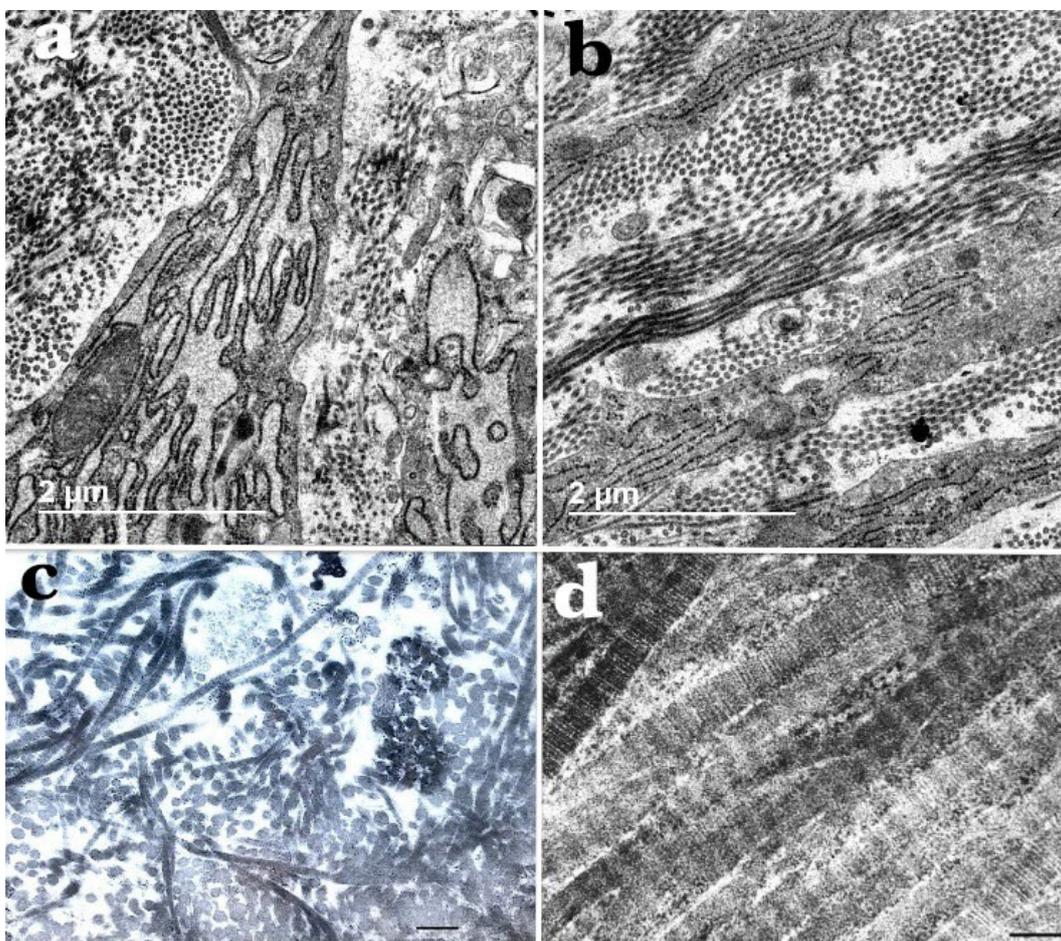


Fig. 14. Collagen appearances in woven and lamellar bone are shown by TEM. (a) TEM in mouse neonatal femoral cortical bone showing lamellar matrix in cross-section adjacent to osteoblast at right. The dilated rough endoplasmic reticulum was a structural sign of active matrix synthesis. Scale bar: 2 μ m. (b) TEM in mouse neonatal femoral cortical bone showing osteoblasts synthesising lamellar bone alternating with bundles of parallel collagen fibres. Cells were elongated, with the long axis of the cells parallel to the lamellar orientation of the collagenous matrix. Parallel fibril groups were also in orthogonal array (at right angles to one another in alternating layers). Surface osteoblasts synthesised collagen fibrils in parallel array. The widely dilated rough endoplasmic reticulum was indicative of active collagen synthesis. Scale bar: 2 μ m. (c,d) TEM showing matrix orientations from tissue samples of patients with osteogenesis imperfecta. (c) Woven bone matrix from a patient with type III osteogenesis imperfecta. Scale bar: 250 nm. (d) Lamellar orientation of collagen fibrils in high magnification in bone tissue from a patient with osteogenesis imperfecta type III. Scale bar: 132 nm.

Table 7. Classification of stages of woven bone formation.

Stage I	Highly cellular, closely packed accumulations of differentiating pre-osteoblasts present <i>de novo</i> at sites with no evidence of pre-existing bone tissue. These pre-osteoblasts have differentiated from the undifferentiated mesenchymal-cell pool.
Stage II	Mesenchymal osteoblasts surround themselves in a 360° arc with randomly oriented matrix fibres: a. cell area is larger than matrix area, b. cell area is equal to matrix area, c. matrix area is larger than cell area.
Stage III	The matrix is sufficient to act as a scaffold on which surface osteoblasts begin to synthesise bone in lamellar conformation.
Stage IV	There is progressive diminution of woven bone in the woven bone/lamellar bone complex. The diminution of woven bone is relative due to the increased synthesis of lamellar bone and absolute due to osteoclast resorption: a. woven bone is more than lamellar bone, b. woven bone is equal to lamellar bone, c. lamellar bone is more than woven bone.
Stage O/stage V	When the entire bone tissue developmental sequence from undifferentiated mesenchymal cell to exclusively lamellar bone is considered, stage O is defined as encompassing mesenchymal cell development from undifferentiated elongated cells to progressively differentiating oval cells, stage V as when all bone seen in a region is lamellar.

tissue between the two; ii) the orientation of the lacunar-canalicular system provides a prominent indication of the matrix conformation it is associated with. Such distinction is valuable as a marker in palaeontology studies. Osteocytes and their lacunae in lamellar bone are elliptical and elongated and lie in the longitudinal plane of the lamellar fibres while their canaliculi are distributed in a uniform fashion passing through the matrix at right angles to the sidewalls of the lacunae. Osteoblasts/osteocytes of woven bone are round to oval and their canaliculi are randomly oriented as they pass through the woven bone matrix. Organisation of the osteocyte network mirrors the extracellular orientation in bone (Kerschnitzki *et al.*, 2011) and osteocyte lacunae and canaliculi are well oriented in relation to the parallel collagen fibres in osteonal bone (Repp *et al.*, 2017). Current studies assess shape modulation of osteoblast-osteocyte transformation and its relationship to matrix conformation (Dallas and Bonewald, 2010; Pazzaglia *et al.*, 2010).

Polarised-light microscopy is essential for studies needing specific identification of woven and lamellar tissues. Human foetal bone, considered by many to be exclusively woven, has considerable amounts of parallel-fibred lamellar bone synthesised as early as 4 months of foetal life (Fig. 5c; 6b,d). Repair bone and pathological osteogenesis imperfecta bone are clearly highlighted by the technique.

Histomorphometry allows for a quantitative assessment of the lacunar-canalicular system. The American Society for Bone and Mineral Research (ASBMR) histomorphometry nomenclature has played a major role in assessing bone structure, with specific indices used for specific functions, such as bone synthesis or resorption (Dempster *et al.*, 2013). Histomorphometry was used to create specific indices to differentiate woven from lamellar bone. A ratio that quantifies the cellularity of woven and lamellar bone helps determining if the amount of

bone synthesised in relation to the cellularity bears any relationship to the recruitment of mesenchymal *versus* surface osteoblasts. When enough woven bone scaffold is present, it favours synthesis of lamellar bone. While further studies are being performed, in normal conditions, a bone index reaching 0.012 is associated with bone deposition in a lamellar pattern whereas an index of 0.024 is associated with lamellar deposition in osteogenesis imperfecta [Maguire *et al.* (2011) Osteogenesis imperfecta bone: correlation of histologic structure and radiographic appearance. American Academy of Orthopaedic Surgeons annual meeting, San Diego, CA, USA; Shapiro and Flynn, 2005].

TEM observations support matrix conformation findings apparent by light microscopy. TEM of decalcified bone demonstrates orientation of the collagen matrix and has helped determine the varying patterns of lamellar tissue orientation, such as orthogonal arrays and twisted plywood arrays (Giraud-Guille, 1988; Raspanti *et al.*, 1996). In lamellar bone there is longitudinal and transverse fibril orientation while in woven bone a characteristic randomly arrayed fibril weave is evident. TEM of calcified sections assesses mineralisation of the collagen fibrils in the differing tissue conformations. Isolated mature apatite crystals (by TEM) are platelet-like in shape, with approximate average dimensions of 50-100 nm or more in length and 25-50 nm in width, while relatively newly deposited crystals, such as in woven bone, are considerably smaller, in the 4-6 nm thickness range (Landis *et al.*, 1993). Mature apatite platelets are oriented approximately parallel to one another and to the collagen fibrils with which they are associated in comparison to human foetal woven bone where there are relational differences (Su *et al.*, 2003). Woven-bone-like nanocomposites have been used in assessing such aspects of bone, such as the role of cross-links in biomineralisation (Li *et al.*, 2012). Collagen fibril orientation in lamellar bone

in particular is well shown by scanning electron microscopy (Boyde and Hobdell, 1969; Frasca *et al.*, 1977; Jones *et al.*, 1975).

Experimental mechanical loading models assess formation of woven bone

Bone tissue differentiation and matrix conformation evolve under a combination of biophysical (mechanical) and molecular influences. An excellent overview has presented the features of tissue adaptation as a dynamic process using woven bone to lamellar bone formation as a prime example (Weinans and Prendergast, 1996). Currey presented the mechanical implications of tissue conformation (Currey, 1984; Currey, 2001; Currey, 2003). Mechanical-loading models in experimental animals have demonstrated that strains initially induce woven bone formation (Burr *et al.*, 1989; Kidd *et al.*, 2010; McKenzie and Silva, 2011a; McKenzie *et al.*, 2011b; Rubin *et al.*, 1995; Turner *et al.*, 1994; Uthgenannt *et al.*, 2007). This is rapidly followed by densification of the woven bone (more woven bone per unit area and increased calcification of the woven bone) and, then, by lamellar bone formation. Interpretation of these findings is that woven bone is a normal adaptive response to applied external mechanical strain, primarily forming on the periosteal surface. Animal experiments involved carefully measured i) strains to intact forearm bones, where the other forearm bone had transverse osteotomy alone or localised resection of bone, or ii) controlled strains that induced bending or stress fracture in one bone but not complete fracture. Mechanical-loading models are sufficiently sensitive that, depending on the extent of the loading, either woven or lamellar bone formation can be induced initially.

Molecular findings accompanying woven and lamellar bone formation

Molecular features of normal bone formation are being increasingly elucidated. These involve not only the composition of the main matrix component, collagen, but also the molecular cascades controlling mesenchymal cell differentiation and osteoblast function. Gorski (1998) pointed out probable molecular differences underlying woven and lamellar bone formation and histological studies confirm this separation of osteoblast functionality. Post-translational assessment of collagen fibrillogenesis has been effectively studied in tissue culture and can be adapted for specific questions (Gerstenfeld *et al.*, 1993). There is an extensive literature on the molecular composition of normal and repair bone formation. Most studies refer to early presence of woven bone and eventual predominance of lamellar bone (Claes *et al.*, 2012; Einhorn and Gerstenfeld, 2015; Franz-Odenaal *et al.*, 2006; Franz-Odenaal, 2011). Certain molecules have been identified as concentrated at certain temporal stages of bone formation and inferred as being associated with

woven (early) or lamellar (later) bone formation without, however, being specifically related to any particular structural conformation. Studies also have identified molecular characteristics of the close interplay between osteogenesis and angiogenesis (Percival and Richtsmeier, 2013). Mechanical loading models inducing bone formation have compared the temporal expression of molecular constituents of woven and lamellar bone. McKenzie *et al.* (2011b) identified 395 genes differentially expressed between woven and lamellar bone 1 h after loading, with 5,883 and 5,974 genes expressed on days 1 and 3, respectively. Microarray analysis grouped these changes into initial inflammatory, followed by vascular response, osteogenesis and bone remodelling genes. These stages are also identified in histological studies outlined in the present review. These studies have demonstrated the ability to correlate histological cell accumulations with molecular concentrations. Many studies have outlined molecular associations with normal and repair bone development at different time periods; although, these studies have not separated woven from lamellar bone. The terms were based on the assumption that earlier bone had higher woven and more mature bone higher lamellar components.

Studies on the molecular nature of woven *versus* lamellar bone would benefit from specific assessments of known molecules that play important roles but are present in extremely small amounts not generally recognisable on gene screening studies. Such possibly important molecules in woven bone formation are some of the “minor” collagens, such as types III and V, and intermolecular collagen crosslinks. Collagen crosslink formation is characterised by developmental changes as collagen matures (Eyre *et al.*, 2008; Knott and Bailey, 1998; Hanson and Eyre, 1996; Rodriguez-Pascual and Slatter, 2016). The crosslinks affect the strength of collagen fibrils (Garnero *et al.*, 2006) as well as the rate and type of their mineralisation (Wassen *et al.*, 2000). No studies have assessed them in relation to woven and lamellar bone, to the authors’ knowledge. Recent technical advances, such as laser capture microdissection, allow localised and specific correlation of structural histological findings with associated molecular components. This methodology has been used extensively in assessing the molecular biology of tumours (Bertos and Park, 2016). Laser-capture microdissection and microarray analysis have also been valuable identifying molecules in the same tissues at different sites. In the distal mouse femur, Shapiro *et al.* (2009b) compared hypertrophic zone chondrocytes of the secondary ossification centre to hypertrophic chondrocytes of the physis at the same structural stage of development and demonstrated differences in the molecular profile. There are specific structural/histological features of woven and lamellar bone as well as a demonstrated sequence of woven to lamellar bone formation. Much valuable understanding can come from localised

molecular assessments of immediately adjacent cell concentrations, such as MOBLS and SOBLS, even though both are defined in newly forming bone as osteoblasts. It will be interesting to determine whether expression of different molecules in the matrices of woven and lamellar bone simply reflect an invariable temporal sequence of initial bone formation (woven) followed by later bone formation (lamellar) or whether the differences are specific for woven bone and lamellar bone conformations, regardless of a temporal sequence.

Conclusions

The ubiquitous presence of woven bone in vertebrates has a great biological significance. Woven bone precedes and induces more mature lamellar bone formation. It has two major functions, serving as an active nidus initiating bone formation *de novo* at sites where no previous bone exists and as a scaffold on which lamellar bone is synthesised. Mesenchymal osteoblasts and early woven bone must contain high concentrations of molecular factors directing cell differentiation into bone-forming lines.

Studies of bone formation increasingly recognise differing osteoblast populations associated with the two matrix conformations, woven and lamellar. It is not known whether these two osteoblast types represent distinct genetic lineages or whether they stem from a single osteoblast lineage that responds differently to differing biophysical stimuli.

Osteoblasts of woven bone, referred to as MOBLS, are surrounded in a 360° arc with randomly arrayed collagen bundles; osteoblasts of lamellar bone, referred to as SOBLS, are aligned linearly on woven bone surfaces (or adjacent pre-existing lamellar bone or metaphyseal calcified cartilage) to synthesise parallel-fibred lamellar bone. While all osteoblasts are derived from mesenchymal cells, the term mesenchymal osteoblast was chosen to indicate initial bone formation *de novo* in a cellular mass where no bone previously existed, as would occur in the mesenchyme of classical embryological terminology. The term surface osteoblast was chosen to describe the striking linear cellular alignment.

The term static osteogenesis describes MOBLS forming woven bone, while dynamic osteogenesis describes SOBLS forming lamellar bone. The two lines of osteoblast function are both active processes resulting from undifferentiated mesenchymal cell movements. MOBLS differentiate from the mesenchymal tissue mass to synthesise woven bone *de novo* where no previous bone existed. SOBLS differentiate from the mesenchymal tissue mass to synthesise lamellar bone becoming positioned in a linear array once a surface scaffold is present.

Woven bone is synthesised more rapidly than lamellar bone.

Bone tissue formation from initial woven to eventual lamellar matrix conformation follows

a repetitive obligate self-assembly pattern in all vertebrates in normal, repair and pathological states.

Synthesis of lamellar bone on woven bone is an abrupt distinct occurrence; gradual intermediate tissue deposition from woven to lamellar bone is not seen.

Six major histological features distinguish woven from lamellar bone.

Lamellar bone structure varies in different vertebrates. In human and some larger mammalian cortices, secondary osteons (Haversian systems) predominate but many species have cortical fibrolamellar or woven-parallel complex bone in plexiform or reticular patterns with some primary but few or no secondary osteons.

The study has allowed for a classification of woven bone development from stage I to stage IV. Stage O was also defined as encompassing mesenchymal cell development from undifferentiated thin, flattened elongated cells to progressively differentiating oval to round shaped pre-osteoblast cells. Stage V defines when all bone in a region is lamellar.

Once woven bone has formed, it quickly serves as a scaffold for synthesis of lamellar bone. Woven bone infrequently exists alone in large accumulations; it invariably becomes covered by SOBLS and parallel-fibred lamellar bone and exists as a woven-lamellar, fibro-lamellar or woven-parallel-fibred complex.

Woven bone is removed by osteoclasts once its role as a scaffold for lamellar bone deposition is completed.

Intramembranous and endochondral bone formation mechanisms synthesise bone that is ultimately lamellar, with the same molecular constituents; the mechanisms differ regarding the scaffold tissue being replaced: woven bone at the cortices (intramembranous) and calcified cartilage (endochondral) at internal marrow trabeculae.

Correlative studies using laser capture microdissection, microarray analysis and other evolving technologies allow for specific isolation of MOBLS/static osteogenesis/woven bone and SOBLS/dynamic osteogenesis/lamellar bone and determination of their gene/molecular profiles.

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Discussion with Reviewers

Daniel Youngstrom: Do mesenchymal and surface osteoblasts represent discrete lineage states or are they functional definitions based primarily on the ultrastructure of the associated bone matrix?

Authors: Mesenchymal and surface osteoblasts are currently functional terms based on their specific and unique association with woven and lamellar bone conformations, respectively. As indicated in Conclusions, it is not known whether these two osteoblast types represent distinct genetic lineages or whether they stem from a single osteoblast lineage that responds differently to differing biophysical stimuli. Isolation of the two groups of osteoblasts for molecular analyses, which is difficult but now technically feasible, should help answer the question.

Daniel Youngstrom: What, if anything, is known about the cell/molecular basis of the abrupt transition from woven to lamellar bone?

Authors: The abrupt transition from woven to lamellar bone is observed invariably using a light microscope and at the ultrastructural level. While woven bone serves as a scaffold for surface osteoblasts, as do calcified cartilage cores in the metaphyses, we are not aware of investigations as to the biophysical or molecular events underlying this occurrence.

Editor's note: The Scientific Editor responsible for this paper was Martin Stoddart.