Review
An overview of the regulation of bone remodelling at the cellular level
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Abstract
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Keywords: Osteoclast, Osteoblast, Osteocyte, Osteomac, Bone lining cell, Bone remodelling, Coupling factors, Endothelial cell, RANKL, Osteoporosis

Objectives: To review the current literature on the regulation of bone remodelling at the cellular level.

Design and methods: The cellular activities of the cells in the basic multicellular unit (BMU) were evaluated.

Results: Bone remodelling requires an intimate cross-talk between osteoclasts and osteoblasts and is tightly coordinated by regulatory proteins that interact through complex autocrine/paracrine mechanisms. Osteocytes, bone lining cells, osteomacs, and vascular endothelial cells also regulate bone remodelling in the BMU via cell signalling networks of ligand–receptor complexes. In addition, through secreted and membrane-bound factors in the bone microenvironment, T and B lymphocytes mediate bone homeostasis in osteoimmunology.

Conclusions: Osteoporosis and other bone diseases occur because multicellular communication within the BMU is disrupted. Understanding the cellular and molecular basis of bone remodelling and the discovery of novel paracrine or coupling factors, such as RANKL, sclerostin, EGFL6 and semaphorin 4D, will lay the foundation for drug development against bone diseases.

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Introduction
Bone is a rigid yet dynamic organ that is characterised as a type of connective tissue. Biochemically, it is defined by a mixture of inorganic...
Bone remodelling is the predominant metabolic process regulating bone structure and function during adult life [1,2]. Remodelling is a complex, tightly regulated process carried out by two key cell types: osteoclasts and osteoblasts. Osteoclasts are the principal resorptive cell of bone, playing a role in the formation of the skeleton and regulation of bone mass. Osteoblasts are specialised bone forming cells that synthesise bone matrix, regulate mineralisation and finally differentiate into osteocytes or bone lining cells. The cellular coupling between the activities of the osteoclasts and osteoblasts is highly regulated by local and systemic factors to maintain bone homeostasis [3]. An imbalance in the bone remodelling process, favouring either osteoclast or osteoblast activity, leads to a number of clinical disease conditions including osteopenia, osteoporosis and osteopetrosis. The exact mechanisms responsible for aberrant bone remodelling are still largely unclear. Therefore, understanding the cellular and molecular mechanisms involved in bone remodelling may provide important insight(s) for therapeutic development.

**The bone remodelling unit**

Bone remodelling occurs through the concerted action of a functional cohort of cells termed the basic multicellular unit (BMU). The BMU consists of the osteoclasts resorbing bone, the osteoblasts replacing bone, the osteocytes within the bone matrix, the bone lining cells covering the bone surface and the capillary blood supply (Fig. 1). The remodelling cycle begins with an initiation phase that includes the recruitment of osteoclast precursors, their differentiation into mature osteoclasts as well as activation and maintenance of bone resorption. A reversal period then follows whereby osteoclastic bone formation is inhibited and the osteoclasts undergo apoptosis whilst osteoblasts are recruited and begin to differentiate. The reversal phase is a transition from osteoclast to osteoblast activity. The final stage is bone formation by the osteoblasts and is termed the termination phase [4]. This stage lasts the longest, as bone formation is slower than bone resorption, and involves new bone formation and mineralisation as well as terminal differentiation of the osteoblast.

The length of the bone remodelling process varies by location, with it being shorter in cortical bone than in cancellous bone. The average length of the remodelling phase in cancellous bone is about 200 days, with the majority of that time (approx. 150 days) devoted to bone formation [5,6].

As bone remodelling is a multicellular event, signalling and cross-talk between the cells involved is important in controlling this process. Recently, progress has been made on the identification of ‘coupling factors’, which coordinate the temporal activation and function of the cells within the BMU. This review focuses on the roles of the cells in the BMU and the interaction between these cells and the factors involved in regulating bone remodelling at the cellular level.

**Osteoclasts**

Osteoclasts are multinucleated, giant cells formed by the fusion of mononuclear progenitors of the monocyte/macrophage family in a process termed osteoclastogenesis [2]. Osteoclasts are located on endosteal surfaces within the Haversian system and on the periosteal surface beneath the periosteum. Osteoclasts are usually rare cells in the bone with only two to three per μm³ [7]. Osteoclasts exist in two functional states, the motile and the resorptive phases. During the motile state they migrate from the bone marrow to their resorptive site and in the resorptive phase they exert their bone resorbing function [8]. In each state the osteoclast displays morphological differences. Motile osteoclasts are flattened, non-polarised cells. They are characterised by the presence of membrane protrusions, called lamellipodia, and podosome complexes containing actin. Osteoclasts move and spread by lamellipodia formation. Upon reaching the resorptive site, osteoclasts become polarised through cytoskeletal reorganisation. Polarisation results in the formation of a number of membrane domains: a ruffled border, sealing zone, functional secretory domain and basolateral membrane. The sealing zone is an osteoclast specific structure which separates the acidic resorptive environment from the rest of the cell, forming an organelle free area [9]. The ruffled border and sealing zone is seen only in non-motile resorbing cells. During resorption osteoclasts are dome-shaped and lack lamellipodia.

**Regulation of osteoclast differentiation in the BMU**

Osteoclast differentiation and fusion requires a number of steps to occur and a number of osteoclastogenic factors regulate these processes.

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*Fig. 1. The basic multicellular unit of bone remodelling. The bone remodelling compartment consists of osteoclasts, osteoblasts, osteocytes, bone lining cells and endothelial cells which work together to ensure balanced bone remodelling. Each cell type produces modulatory factors that regulate other cell types and some are highlighted in this figure.*
Haematopoietic stem cells give rise to osteoclast precursors which are a subset of bone marrow mononuclear cells. These cells require factors produced either by marrow stromal cells, osteoblasts or T-lymphocytes to differentiate further [10]. Two such factors, which are necessary and sufficient to promote osteoclastogenesis, are macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor kappa B (NF-κB) [RANK] ligand (RANK).

M-CSF, produced by osteoblasts and stromal cells, is critical for macrophage maturation and binds to its receptor, c-fms, on early osteoclast precursors thereby promoting their survival and proliferation [11,12]. Signal transduction via the c-fms receptor occurs through multiple pathways, including ERK1/2 and PI3K/Akt [13]. M-CSF activates early transcription factors such as c-Fos and PU.1. Mice deficient in M-CSF or PU.1 lack osteoclasts and macrophages whilst the inactivation of c-Fos results in arrested osteoclastogenesis and an osteopetrotic phenotype [14,15]. It is interesting to note though, that the osteopetrotic op/op mouse, which has a mutation resulting in the absence of M-CSF, restores osteoclast differentiation and function over time, suggesting that other factors may be able to compensate for the loss of M-CSF [16,17].

RANKL, expressed by osteoblasts, T cells and endothelial cells, is essential for osteoclast formation and binds to RANK which is present on osteoclasts and their precursors [18–20] (Fig. 2). RANKL stimulates the M-CSF-expanded precursors to commit to the osteoclast phenotype. These cells then express key osteoclast markers including tartrate resistant acid phosphatase (TRAP). Upon further stimulation with M-CSF and RANKL, the preosteoclasts fuse to form multinucleated cells which begin to express more specific osteoclast markers such as calcitonin receptor and Cathepsin K. The binding of RANKL to RANK is dependent on trimerisation of both molecules [21]. RANKL activity can be antagonised by the presence of the soluble decay receptor Osteoprotegerin (OPG) [22]. This sequesters RANKL from binding to RANK and inhibits osteoclast differentiation. The transmission of the RANKL signal is determined by the ratio of RANKL to OPG, coupled with RANK expression on osteoclast precursors. Like RANKL, OPG is produced by osteoblasts, giving osteoblasts a vital role in controlling the relationship between bone formation and bone resorption [23,24].

Binding of RANKL to its receptor initiates an intracellular signalling cascade that begins with binding of TRAF6 to the RANK receptor [25] (Fig. 2). TRAF6 then phosphorylates IKK (IκB kinase) which ubiquitinates IκB, sending it to the proteasome for degradation. This releases NF-κB, allowing its translocation to the nucleus and the initiation of osteoclast specific gene transcription. Abnormal activation of NF-κB signalling pathways in osteoclasts disrupts bone remodelling and results in osteolytic bone diseases [26].

Although binding of RANKL has been demonstrated to be the essential signal for osteoclast differentiation, co-stimulatory pathways are also required for this process. The transcription factor nuclear factor of activated T cells (NFAT) has been shown to play an important role in osteoclast differentiation [27] (Fig. 2). RANKL stimulates calcium oscillation which is essential for activation of NFAT. This occurs through co-stimulation of immunoglobulin like receptors such as OSCAR (osteoclast associated receptor) and TREM-2 (triggering receptor expressed on myeloid cells), and the function of adaptor molecules containing ITAM motifs [28]. This signalling cascade activates phospholipase Cγ (PLCγ) and subsequent intracellular Ca2+ release, leading to the nuclear translocation of NFATc1 and subsequent transcription and autoamplification of NFATc1. In addition, a role for CaMK (calmodulin kinase), a major downstream mediator of Ca2+ signalling, plays an important role in RANKL induced osteoclast differentiation and bone resorption [29,30].
Role of osteoclasts in bone remodelling

Although the primary function of the osteoclast in bone remodelling is resorption of the bone matrix, it also functions in the regulation of bone formation. This can be achieved by secretion of factors that regulate bone formation, liberation of matrix derived factors during resorption or direct cell–cell contact with osteoblasts (Fig. 3).

The bone resorbing activity of osteoclasts is one way in which bone formation is regulated [31]. The topography of the resorption pit itself is an important factor for the induction of bone formation [32]. In the absence of osteoclasts, osteoblasts may recognise parts of the resorption lacuna such as the deposition of TRAP, which is required by osteoclasts for bone matrix resorption and collagen turnover [33,34]. RANKL expressed by osteoclasts can also directly activate osteoclastic bone resorption, in addition to its role in osteoclast formation [19,20].

As the osteoclast resorbs bone it releases bone matrix-derived factors that may regulate osteoblastic bone formation. These factors are often produced by osteoblasts at the time the bone matrix is laid down and act on the osteoblasts to promote bone formation; however, it is likely that as the osteoclast resorbs bone it processes these bone matrix factors and regulates the amount released to act on the osteoblasts. Such factors include bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs) I and II and transforming growth factor beta (TGF-β) [35–37]. However, osteoclast resorptive activity is not essential for osteoblastic bone formation to occur as patients and mice with osteopetrosis due to impaired osteoclast function (osteoclast-rich osteopetrosis) still have high numbers of functional osteoblasts indicating that it is the number of osteoclasts, rather than their functional activity, that is more crucial for bone formation [38–41]. It is also important to note that osteoclast poor osteopetrotic patients are also able to form bone, suggesting that osteoclasts are not essential to the fundamental process of bone formation [42,43].

The possible ways in which osteoclasts can regulate osteoblasts has been a topic of great interest and much work has been carried out to identify coupling factors that represent potential therapeutic targets. Sphingosine 1-phosphate (S1P) is secreted by osteoclasts and binds to its receptor on osteoblasts enhancing migration and survival of osteoblasts as well as increasing RANKL production [44]. S1P also has direct effects on osteoclasts by regulating the migration of osteoclast precursors [45]. Ephrin B2, a membrane bound ligand expressed on osteoclasts, binds with its receptor EphB4 on osteoblasts, in a contact dependent mechanism. This interaction promotes osteoblast differentiation and bone formation [46]. The ephrin/Eph interaction results in bi-directional signalling meaning that EphB4 can signal to the osteoclast to suppress differentiation [47]. Since ephrin B2 is expressed by mature osteoclasts and EphB4 is expressed on osteoclast precursors it has been hypothesised that this interaction may regulate the transition from bone resorption to formation during remodelling. Most recently, semaphorin 4D has been identified as a coupling factor. Semaphorin 4D is expressed by osteoclasts and inhibits bone formation by binding to its receptor Plexin-B1 on osteoblasts. This interaction activates RhoA which in turn suppresses insulin like growth factor 1 (IGF-1) signalling and modulates osteoblast motility, thereby inhibiting bone formation [48].

Osteoblasts

Osteoblasts are the only cell type responsible for bone formation. They originate from mesenchymal stem cells that have the potential to differentiate into mature osteoblasts [49,50]. There are four maturation stages that have been identified in osteoblast differentiation: the preosteoblast, osteoblast, osteocyte and bone-lining cell. Provided the appropriate stimuli are present, the mesenchymal stem cells form preosteoblasts. Histologically these cells resemble osteoblasts and stain positively for alkaline phosphatase, however, they lack some of the characteristics of mature osteoblasts including the ability to produce mineralised tissue [51]. Preosteoblasts give rise to mature osteoblasts, which can be identified by their cuboidal morphology and strong alkaline phosphatase positivity. They reside along the bone surface at sites of active bone formation. Osteoblasts secrete type 1 collagen, the basic building block of bone. In addition, they produce non-collagenous proteins including osteocalcin (a small vitamin-K dependent calcium binding protein that is specific to bone) and alkaline phosphatase, which is essential for mineral deposition [52]. Alkaline phosphatase knockout mice exhibit impaired growth and die before weaning. Examination of the tissues revealed defective mineralisation and morphological abnormalities in the osteoblast highlighting the importance of alkaline phosphatase for osteoblast function [53].

Mature osteoblasts have one of three fates: they undergo apoptosis, differentiate further into osteocytes or become quiescent lining cells. Approximately 50 to 70% of osteoblasts undergo apoptosis [54].

One of the most important signalling pathways regulating bone formation in osteoblasts is the Wnt/β-catenin signalling pathway. The canonical pathway has been most studied and is illustrated in Fig. 4. Wnt ligands bind to a receptor complex composed of frizzled receptor and LDL receptor related protein (LRP) 5 or LRP6 co-receptor. In the absence of ligand binding, glycogen synthase kinase (GSK)-3β acts to phosphorylate β-catenin leading to its ubiquitination and subsequent proteasomal degradation. Binding of Wnts to their receptor complex, leads to the activation of Dishevelled, which inhibits the activity of GSK–3β. This prevents the degradation of β-catenin, which is then able to translocate to the nucleus where it

![Fig. 3. Mechanisms of osteoblast/osteoclast regulation. Osteoblasts and osteoclasts regulate one another via several mechanisms including: (i) bone matrix derived factors that are released during resorption and act on both osteoclasts and osteoblasts, (ii) direct cell–cell contact between osteoclasts and osteoblasts and (iii) secretion of factors by the osteoclast that regulates the osteoblast and vice-versa.](image-url)
activates LEF (lymphoid enhancer factor)/TCF (T-cell factor) mediated gene transcription [55–57]. Various physiological inhibitors of the Wnt/β-catenin pathway exist and they function predominantly by preventing formation of the receptor/ligand complex. These include sclerostin and Dickkopf (Dkk)-1, which bind to LRPs/5, and secreted frizzled related protein (Sfrp) which binds to Wnt [56,57]. The importance of the Wnt/β-catenin pathway in bone biology is highlighted by the effects of mutations in the LRPs gene in humans. Loss-of-function mutations in LRPs exhibit low bone mass due to altered osteoblast proliferation and function [62]. Mice overexpressing LRPs in osteoblasts have increased bone mass [63]. For a detailed review on animal studies involving genetic manipulation of various molecules in the Wnt/β-catenin pathway refer to Kubota et al. [57].

Other important signalling pathways involved in osteoblast differentiation and bone formation include those of the TGF-β superfamily, the two key members being TGF-β and BMPs (for a detailed review on TGF-β and BMP signalling see [64]). TGF-β signalling is essential for the maintenance and expansion of osteoprogenitor cells and their commitment to the osteoblast lineage. This is achieved through the activation of selective mitogen activated protein kinases (MAPKs) and Smad2/3 signalling [65,66]. The BMP signalling pathway also requires the activation of Smads and their subsequent translocation to the nucleus to activate the transcription of osteogenic genes. The TGF-β and BMP signalling pathways interact with one another as TGF-β1 enhances bone formation induced by BMP2 [67]. Both pathways also interact with the Wnt, PTH and Notch pathways to name a few [68].

**The role of osteoblasts in bone remodelling**

**Bone formation**

The primary function of the osteoblast is bone formation. Therefore, the osteoblast has a role in the initial formation of the skeleton as well as in continued bone growth and remodelling processes. Bone formation occurs via two distinct mechanisms: intramembranous ossification and endochondral ossification. Intramembranous ossification forms the flat bones of the skull and most of the clavicle. This type of bone formation relies on a pre-existing fibrous layer of connective tissue onto which the osteoblasts deposit organic matrix composed of Type 1 collagen. Calcium phosphate is then deposited in the matrix forming spongy bone. In contrast, endochondral ossification, which produces most bones, involves the transformation of mesenchyme into a cartilage model that resembles the shape of the bone. The cartilage is then invaded by blood vessels and gradually broken down as osteoblasts deposit collagenous organic matrix and regulate mineralisation of the matrix by releasing small membrane-bound vesicles that contain calcium and phosphate [69]. The exact mechanism by which mineralisation occurs remains unclear; however, it is thought that the activity of alkaline phosphatase plays an important role [70]. Hyaline cartilage remains on the epiphyseal surfaces where it forms articular cartilage and at the junctions of the diaphysis and epiphysis where it forms the growth plate, allowing the long bones to extend in length during growth.

**Regulation of the osteoclast**

Besides their role in bone formation, osteoblasts play an important role in the differentiation of osteoclasts. The regulation of osteoclasts by osteoblasts occurs via factors deposited in the bone matrix which osteoclasts then release during resorption, secretion of factors that act on osteoclasts or direct cell–cell contact (Fig. 3). These are similar to the ways in which osteoclasts regulate osteoblasts.

Osteoclasts produce M-CSF, RANKL and OPG. As discussed above, these factors are essential for osteoclast formation and function. RANKL is the master cytokine for osteoclast differentiation whilst OPG acts as a decoy receptor controlling the amount of RANKL activating osteoclasts [71]. In addition to regulating osteoclast differentiation, osteoclasts control the movement of osteoclast precursors to the bone surface through the release of chemoattractants. Two bone matrix-derived chemoattractants are osteocalcin and collagen-1 [72]. These are produced by the osteoblasts and are deposited in the matrix during bone formation. As osteoclasts resorb bone these chemotactants may be released, thereby attracting more osteoclast precursors to the remodelling site to continue resorption. As described below, the bone lining cells may also assist in the release of bone-matrix derived chemoattractants. Monocyte chemoattractant protein (MCP-1) is produced by osteoblasts and recruits osteoclasts.
precursors. The expression of the MCP-1 receptors on osteoclasts is induced by RANKL, indicating that osteoclasts can control the MCP-1 dependent recruitment of osteoclast precursors by regulating RANKL expression [73].

There are a number of other factors that indirectly regulate osteoclastogenesis by acting on osteoblasts. Such soluble factors include parathyroid hormone (PTH), parathyroid hormone related peptide (PTHrP), tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and 1,25-(OH)2 vitamin D3 to name a few [74–78]. These factors act on osteoblasts to increase RANKL expression, thereby increasing osteoclast differentiation. Some of the factors can act through a dual capacity and are able to inhibit production of OPG by osteoblasts [79]. Osteoblasts are able to regulate their differentiation through the paracrine factor PTHrP. PTHrP is produced by preosteoblasts and acts via the PTH1 receptor present on osteoblasts to stimulate osteoblast differentiation and inhibit osteoblast apoptosis [75,80]. PTHrP therefore has a dual role in regulating osteoclastogenesis and osteoblast differentiation.

Osteoblasts play a major role in regulating osteoclast differentiation but whether they control osteoclast activity is not so clearly known. They may play a role indirectly when they lay down the bone matrix through the secretion of matrix proteins containing the RGD (arginine-glycine-aspartic acid) peptide. For osteoclasts to resorb they are required to attach to the bone surface. This occurs via the interaction of integrins with the RGD peptides in matrix proteins such as osteopontin and bone sialoprotein [81].

**Osteocytes**

Osteocytes are terminally differentiated osteoblasts that incorporate into the newly formed bone matrix [82]. These cells are smaller than osteoblasts and have lost many of their cytoplasmic organelles [83]. Osteocytes lie within lacunae in the newly formed bone matrix where they reside for long periods of time but ultimately undergo apoptosis. Osteocytes are spatially isolated from one another; however, they extend long filipodial extensions, which are rich in actin cytoskeleton, connecting them with each other as well as bone lining cells and osteoblasts on the bone surface [84,85]. There is emerging evidence to suggest that the primary function of the osteocyte relates to the determination and maintenance of bone structure. Osteocytes are mechano-sensors capable of transducing musculoskeletal derived mechanical input into biological output [86]. Microdamage (small deare mechano-sensors capable of transducing musculoskeletal derived evidence to suggest that the primary function of the osteocyte relates regulating the bone's response to mechanical unloading and its expression to the mechanical environment. Sclerostin plays an essential role in and the bone remodelling process.

Sclerostin, the osteocyte-secreted bone formation inhibitor, is thought to be another downstream mediator of skeletal responses to the mechanical environment. Sclerostin plays an essential role in regulating the bone’s response to mechanical unloading and its expression is up-regulated in patients with disuse-induced bone loss [90,91]. It is hypothesised that sclerostin travels through the osteocytic network to the bone surface where it inhibits Wnt/β-catenin signalling in osteoblasts thereby inhibiting proliferation, impairing mineralisation and enhancing apoptosis [92,93].

**Bone lining cells**

A subset of osteoblasts will differentiate into bone lining cells. These cells line the majority of bone surfaces that are not being remodelled. It has been proposed that bone lining cells play a role in bone remodelling by preventing the inappropriate interaction of osteoclast precursors with the bone surface. It is thought that the signals that initiate osteoclast formation may stimulate the bone lining cells to prepare for bone resorption, through the actions of collagenase which digests a thin layer of non-mineralised bone, revealing the mineralised matrix underneath [95,96]. The bone lining cells then migrate to form a canopy over the remodelling area, creating a microenvironment for the coupling required during bone remodelling [97].

It has been a topic of much debate as to how cell associated RANKL on active osteoblasts can direct osteoclast formation and function since osteoblasts form and produce bone in the remodelling site after osteoclast formation and activity has been initiated. It has been proposed that the bone lining cells, which have been shown to express RANKL and other osteoblastic markers when lining active remodelling compartments, are responsible for the cell to cell interactions between RANKL and RANK receptor on osteoclast precursors [97]. This argument holds true for many other proposed coupling factors that are thought to act through direct cell to cell interaction. It is not clear what the likelihood of physical interactions within the narrow compartment and the concentration of factors required to activate bone remodelling processes would be in vivo. Some suggest that the compartmentalisation of the remodelling site through the canopy of bone lining cells serves to localise the required cellular precursors, and the factors released from the resorption site, within a volume that allows for direct cellular interactions and high concentrations of cytokines that might otherwise be diluted and rendered ineffectual within the bone marrow milieu [98].

**Osteomacs**

Osteomacs are resident tissue macrophages present on or near the periosteal and endosteal surfaces. They are located within three cells of a bone surface and are often intercalated or associated with bone lining cells. During bone modelling osteomacs form a canopy-like structure over mature osteoblasts [99,100]. Osteomacs regulate osteoblast mineralisation as evidenced by in vitro and in vivo studies showing that depletion of osteomac populations results in reduced osteoblast mineralisation [100]. This suggests that the osteomac population plays a role in bone homeostasis.

Since osteomacs are located in close proximity to osteoblasts and regulate osteoblast mineralisation, it has been proposed that osteomacs may provide a coupling signal to osteoblasts during bone modelling in a similar way that osteoclasts provide coupling signals to osteoblasts during bone remodelling [101]. TGFβ and ephrin B2 are potential coupling signals since they are expressed by osteomacs and have been previously implicated in osteoclastic regulation of bone formation. A recent study has shown that osteomacs enhance intramembranous bone formation during bone healing in a tibial injury model [102]. Since intramembranous ossification does not involve resorption of cartilage or a bone template prior to bone formation it has been proposed that the osteomac may serve as a cellular source for the anabolic factors that regulate osteoblast recruitment, maturation and bone formation during this process.

Whether osteomacs play a role in bone remodelling is not as clear. A canopy of osteomacs has been identified in the bone remodelling compartments in mice [101]. Further, Pettit et al. have proposed...
that the osteoclasts may regulate the tail end of the remodelling cycle by directing ongoing and terminal osteoblast mineralisation, once the osteoclasts have left the bone remodelling site [101].

Vascular endothelial cells

A vital, but often overlooked, component of the BMU is the capillary that supplies oxygen and nutrients, and removes calcium and waste products of resorption. Bone is one of the most highly vascularized tissues and angiogenesis plays a pivotal role in bone formation, remodelling and healing [98,103]. The blood supply to bone is essential for maintenance of bone mineral density and bone structure. Pathological situations resulting in loss of the bone blood supply lead to bone death, such as avascular necrosis. Lack of bone vascularity is associated with decreased bone formation and bone mass, inhibition of blood flow to bone inhibits bone healing in fracture models and increases the risk of non-union [103,104].

Bone remodelling takes place in specialised vascular structures. The vasculature provides a conduit for the delivery of systemic signals regulating calcium homeostasis, providing feedback control of the remodelling process directly to the cells involved. One of the most important nutrients transported via the vasculature to the BMU is oxygen, which is fundamental to many cellular processes, in particular energy production. However, there are specific roles for oxygen in relation to bone remodelling. One of the major enzymes required for collagen production is prolyl-4-hydroxylase, which is dependent on molecular oxygen for its function. In the absence of oxygen, osteoblasts cannot produce collagen effectively and their proliferation is reduced [105]. Cellular responses to changes in oxygen tension are directed through the activity of the hypoxia inducible factor (HIF), which is capable of activating gene transcription in response to low oxygen levels [106]. Osteoblast specific knockdown of HIF1α or HIF2α has demonstrated important roles for HIF in controlling bone formation and vascularity [107]. Furthermore, low oxygen environments encourage osteoclast HIF1α stabilisation leading to increased osteoclast number [108–110]. HIF1α has recently been identified as a negative regulator of the osteoblast response to mechanical load. Taken together, endothelial cells and the vasculature have a direct role in bone remodelling as they supply nutrients to the BMU.

It has been widely speculated that bone remodelling is influenced by the local bone environment that involves cross-talk between endothelial cells and adjacent bone cells [98,103]. Vascular endothelial growth factor (VEGF) has long been associated with bone remodelling. Mice expressing only the VEGF 120 isoform survive to adulthood [107]. Expression of RANKL has also been demonstrated in bone-derived vascular endothelial cells and RANKL is upregulated in response to TGFβ, TNFα, and IL-1α [117,118]. Cytokine activated human vascular endothelial cells have been shown to be capable of promoting the formation, fusion and activity of osteoclasts [117,119]. This suggests that the production of RANKL by endothelial cells can have both autocrine and paracrine modes of action. Recent studies have shown that osteoblast like cells produce secreted epidermal growth factor like-6 (EGFL6) that regulates endothelial cell migration and angiogenesis via a paracrine mechanism [120]. Many other factors that are known to be angiogenic are produced by cells in the BMU; further studies into novel regulators of angiogenesis produced by bone cells will give important insight into the cross-talk between the endothelial cells, osteoblasts and osteoclasts.

Lymphocytes — T cells and B cells

The relationship between the immune and skeletal systems has been studied since the early 1970s and as such the term osteoimmuno-nology was created to describe the overlap between these fields (for a detailed review on osteoimmunology see [121]). Given that immune cells and haematopoietic cells originate in the bone marrow it is not surprising that there is cross-talk between the two systems. A number of factors secreted by immune cells are also known to be osteoclast activating such as RANKL secreted by T cells [122,123]. However, the role of immune cells in the maintenance of normal bone physiology is less clearly understood. It is thought that T cells may have a protective role against bone turnover as in vitro studies suggest that CD8+ T cells inhibited osteoclastogenesis [124,125]. Further, when bone marrow derived from CD4+ and CD8+ deficient mice was cultured in the presence of 1,25- (OH)2 vitamin D3 osteoclastogenesis was enhanced, indicating that T cells suppress osteoclast formation [126]. Studies of B cell and T cell deficient mice have highlighted the role of these lymphocytes in bone homeostasis and the attainment of peak bone mass. B cell deficient mice are osteopenic due to increased bone resorption. The deficiency in B cells leads to a deficit in OPG as B cells are a major source of OPG in the bone microenvironment [127]. T cell deficient nude mice also display a decreased basal bone mass phenotype as a consequence of enhanced bone resorption. In nude mice OPG secretion by B cells was reduced in the bone marrow suggesting that T cell deficiency affects B cell OPG secretion [127]. Therefore, in physiological bone homeostasis, T cells play a protective role. Although lymphocytes are not directly present in the remodelling compartment, through the secretion of factors into the bone microenvironment they play a role in bone homeostasis.

The role of the immune system in pathological bone conditions has been more comprehensively studied, mostly due to the fact that autoimmune conditions such as rheumatoid arthritis (RA) have extensive bone destruction. Early studies identified osteoclast-like giant cells at the interface between synovium and bone, suggesting that osteoclasts were responsible for the bone destruction [128]. Further studies showed that synovial fluid contained osteoclast precursor cells and osteoclastogenesis supporting cells confirming the presence of osteoclasts in rheumatoid joints [129]. RANKL is also highly expressed by T cells and synovial cells in the synovium of RA patients [130–132]. Other inflammatory cytokines such as IL-1, IL-16 and TNFα are present in the synovial fluid and are able to induce RANKL expression [121]. In recent times there has been much interest in the type of T cell participating in the enhanced osteoclastogenesis. Tγδ17 (T helper) cells have been identified as the osteoclastogenic T cell subset. These T cells produce IL 17, do not produce large amounts of interferon gamma, express RANKL and trigger local inflammation — key characteristics of osteoclastogenic T cells [133,134]. These T cells are not solely responsible for the osteoclastogenic effect in RA as they are not able to induce osteoclastogenesis in a coculture system [134].

Further understanding of the role of lymphocytes in bone biology as well as the molecules involved in the osteoimmune system may be important for the development of targeted therapeutics for bone diseases.

Diseases due to aberrant bone remodelling

The maintenance of bone homeostasis is dependent on the balance of cellular activities during bone remodelling. Disruption to the
balanced regulation of bone remodelling results in a number of disease conditions including osteoporosis, osteopetrosis and Paget's disease of bone.

Osteoporosis

Osteoporosis is characterised biologically by the net loss of bone, which results in a decrease in bone density. Ultimately, osteoporosis leads to bone with less tensile strength and significantly greater susceptibility to fracture with less force [135]. There are usually no signs or symptoms of osteoporosis until a fracture occurs, which is why osteoporosis is often called the 'silent disease'.

Primary osteoporosis most often occurs in postmenopausal women [135]. This is due to a deficiency in oestrogen that affects circulating levels of cytokines such as IL-1, TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF) and IL-6. As the levels of oestrogen decrease, the levels of these cytokines increase thereby enhancing bone resorption by increasing the recruitment, differentiation and activation of osteoclasts [136,137]. This is mediated by RANKL generated from osteoblasts and other cell types, leading to increased bone resorption [138].

Osteoporosis is also associated with the normal ageing process. A decline in the number and activity of osteoblasts is seen without a decline in the activity of osteoclasts. Thus, a net bone loss is observed due to decreased bone formation [139]. Secondary osteoporosis is often due to complications in other medical conditions, consequences of changes in physical activity or adverse results of therapeutic interventions for certain disorders [140].

Treatment for osteoporosis aim to reduce the risk of further fractures. Anti-resorptive agents, such as bisphosphonates, and the selective oestrogen-receptor modulator raloxifene reduce the intensity of bone remodelling. Slower remodelling permits more complete secondary mineralisation giving the bone more strength. However, these treatments do not reverse the structural damage [141]. The anabolic agent parathyroid hormone produces some reconstruction of the bone skeleton through the deposition of new bone tissue. The overall effect of this is to increase bone strength and reduce fracture risk [142]. In addition to these treatments calcium and vitamin D supplements can be given. Calcium reduces the rate of bone loss and therefore is often used in conjunction with other treatment forms particularly if a patient's dietary calcium intake is low [143]. More recent therapeutic developments have focused on modulating specific steps in the bone remodelling process such as reducing bone resorption and increasing bone formation. Denosumab, a recombiant fully human monoclonal RANKL specific antibody, is an example of one such therapy. Clinical trials indicated that Denosumab effectively suppresses bone resorption, decreases bone turnover and increases bone mineral density [144–146]. The Food and Drug Administration in the US and the European Medicines Agency in Europe have approved Denosumab for clinical use. Emerging data from these clinical trials indicates a promising new treatment for postmenopausal osteoporosis.

Osteopetrosis

Osteopetrosis is defined as a group of conditions characterised by increased bone density as a result of defects in either osteoclast differentiation or function. At least ten mutations have been identified as being causative in humans. Mutations in RANKL and RANK genes result in rare, osteoclast-poor forms of autosomal recessive osteopetrosis (ARO) where no osteoclasts are present [42,43]. Defects in components of the acidification machinery constitute the majority of osteoclast-rich forms of osteopetrosis. Mutations in TCIRG1 (α3 subunit of V-ATPase) are responsible for ARO in more than 50% of affected patients, highlighting the importance of the V-ATPase proton pump in osteoclast function [38]. Chloride channel 7 mutations cause ARO with neuronal complications [147]. Whilst mutations in carbonic anhydrase type II (CAII) result in ARO with tubular acidosis which is not surprising given the role of CAII in kidney function [148]. All the mutations identified so far only account for approximately 70% of cases. Therefore the search continues to find mutations in novel genes. In patients with TCIRG1 gene mutations it was observed that the osteoblasts were very active [149]. This, together with reports that hyperactive osteoblasts were seen in human and osteopetrotic animals, suggests that osteoblasts could contribute to the increased bone mass [150,151].

Current treatment for ARO relies on total bone marrow transplant, although this is not suitable in patients with RANKL mutations. Rather, treatment is largely supportive and relies on multidisciplinary surveillance.

Paget's disease of bone

Paget's disease of bone (PDB) is the second most common bone disorder diagnosed after osteoporosis. It is characterised by areas of increased osteoclast activity and abnormal bone remodelling. The most common symptoms include pain, bone enlargement and deformity, fractures and deafness. PDB is highly localised with affected either in one bone or multiple bones [152]. The most serious complication of PDB is the development of osteosarcomas in the pagetic bone although this is rare with 1% of patients affected [153].

PDB begins as a focal area of bone resorption, and is generally considered a disease of the osteoclast. The osteoclasts in PDB are increased in size and have a large number of nuclei (up to 100) [154]. An unusual feature seen in the pagetic osteoclasts is the presence of nuclear inclusions which consist of paracrystalline arrays that are similar to the nucleocapsids of paramyxoviruses [155]. In addition to these morphological features, pagetic osteoclast precursors are physiologically abnormal. They respond hyper-sensitively to osteoclastogenic factors including RANKL and 1.25-(OH)2 vitamin D3 [156,157]. As is seen during physiological bone remodelling, following bone resorption there is an increase in bone formation with a large number of hyperactive but morphologically normal osteoclasts. The osteoblasts deposit bone in a disorganised manner and therefore the new bone is of a poor quality. During the advanced stages of PDB, bone formation predominates and sclerotic lesions are seen with fibrous tissue deposited in the bone marrow and thickening of the bone observed.

PDB clearly represents a gross distortion of the remodelling process with aberrant coupling between resorption and formation.

The initiation event behind PDB is unknown; however, a genetic cause is strongly implicated. Laurin et al. identified a point mutation in SQSTM1, a gene encoding sequestosome 1 also known as p62, in patients with PDB [158], p62 is a ubiquitin binding protein that is involved in the IL-1, RANKL and TNF signalling pathways. Other mutations have since been identified in the SQSTM1/p62 gene including mutations that result in the complete deletion of the ubiquitin binding domain of p62 [159,160]. SQSTM1/p62 mutations are associated with increased osteoclastogenesis and NF-kB activation [160, 161]. Taken together, SQSTM1 is the gene most frequently linked to PDB however researchers are studying other possible genes that increase or activate osteoclasts. No cure exists for PDB; however, the disease can be controlled through the use of bisphosphonates. Identification of novel genes, such as SQSTM1, which affect the balance of the remodelling process offer new pathways to explore the coupling between bone cells, and new hope for therapeutic discoveries.

Conclusion

The process of bone remodelling is tightly coordinated by a myriad of cellular activities. Whilst intimate cross-talk between osteoblasts and osteoclasts is an integral element in bone remodelling, other cells including osteocytes, bone lining cells, osteomacs and endothelial cells of the basic multicellular unit also play an important

part. An imbalance of these cellular activities often contributes to bone diseases such as osteoporosis and osteoarthritis or leads to bone growth deformities. These conditions are highly significant problems as they cause much morbidity and mortality. A better understanding of the bone remodelling process and the genes involved is vital to improving current options for treating bone diseases. Although the co-ordination of bone remodelling is attributed to bone multicellular signalling networks, there is limited knowledge regarding key bone coupling factors. Thus, deciphering the molecular basis of bone remodelling is of great interest and significance in bone biology.

Conflict of interest

No conflict of interest.

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