A theoretical study of bone remodelling under PEMF at cellular level

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Pulsed electromagnetic field (PEMF) devices have been used clinically to slow down osteoporosis and accelerate the healing of bone fractures for many years. However, the underlying mechanism by which bone remodelling under PEMF is regulated remains poorly understood. In this paper, a mathematical model of bone cell population of bone remodelling under PEMF at cellular level is developed to address this issue for the first time. On the basis of this model and control theory, parametric study of control mechanisms is carried out and a number of possible control mechanisms are identified. These findings will help further the understanding of bone remodelling under PEMF and advance therapies and pharmacological developments in clinical trials.

Keywords: mathematical; bone remodelling; PEMF; cellular

1. Introduction

Pulsed electromagnetic field (PEMF) devices have been widely used clinically for bone healing, muscle relaxation and osteoarthritic joints for many years. The osteogenetic effect of PEMF devices is of great significance to patients, especially those who have undergone failed surgical intervention (Gossling et al. 1992).

The use of electrical stimulation in bone can be dated back almost 200 years ago to when a patient with tibia non-union was successfully cured in 1812 (Brighton and Magnusson 1985). Fukada and Yasuda (1957) discovered the piezoelectrical feature of bone, in that when bone was under compression an electronegative potential was induced, whereas an electropositive potential was produced by bone under tension. Fukada (1982) hypothesised that the growth of bone was regulated to best resist external force, and the controlling signal seemed to be the electric potential generated by shear piezoelectricity in collagen fibres and/or streaming potential in canaliculae (Grande et al. 1991). These two discoveries raised the possibility that the behaviour of bone cells could be affected by externally applied electrical stimuli (Bassett and Pawluk 1964). Bassett (1982) was the first to use a pair of Helmholtz coils to produce a magnetic field across a fracture site and enhance osteogenesis. Qin et al. (Qin and Ye 2004, Qin et al. 2005, Qin 2007) and Qu et al. (Qu and Qin 2006, Qu et al. 2006) studied multifield bone remodelling processes extensively, using the concept of adaptive piezoelectric theory. Recently, several major forms of electrical stimulation have been reported to produce osteogenesis, including capacitively and inductively coupled electromagnetic and direct current fields (Mammi et al. 1993; Kubota et al. 1995). Since then, research into electrically induced osteogenesis in bone has been carried out using these methods both in vivo and in vitro (Kubota et al. 1995; Fredericks et al. 2000; Chang et al. 2004; Sun et al. 2009). The osteogenesis effect on bone can be used not only on long bone fractures (Bassett 1982) but also in osteoarthritic joints (Trock et al. 1994) and osteoporotic bone (Chang and Chang 2003), as well as in reversing femoral head necrosis and augmenting spinal fusion (Linovitz et al. 2002).

The biological process involved in the osteogenesis of bone caused by PEMF devices is known as bone remodelling. At cellular level, bone remodelling is an organised process, where osteoclasts remove old bone and osteoblasts replace it with newly formed bone. The osteoclasts and osteoblasts work in a coupled manner within the so-called basic multicellular unit (BMU), which is a mediator mechanism bridging individual cellular activity to whole bone morphology (Frost 1986) and which follows an activation–resorption–formation sequence (Robling et al. 2006). Since the discovery of the RANK–RANKL–OPG pathway (Anderson et al. 1997), we have a clearer picture regarding the control of osteoclastogenesis and bone remodelling in general. The main switch for osteoclastic bone resorption is the RANKL (Zaidi 2007), a cytokine that is released by preosteoblasts (Pivonka et al. 2008). Its action on the RANK receptor is regulated by OPG, a decoy receptor, which is also derived from osteoblastic lineage active osteoblasts (OBA; Pivonka et al. 2008). Osteoclast-to-osteoblast crosstalk occurs mostly
through growth factors, like transforming growth factor-β (TGF-β), which are released from the bone matrix during resorption. The RANK–RANKL–OPG signalling pathway between osteoblasts and osteoclasts, TGF-β1, PTH and the dual action of TGF-β is diagrammed in Figure 1.

Bone cell differentiation and proliferation are important factors during bone remodelling, and clinical PEMF devices have been shown to affect differentiation and proliferation of bone cells in vitro (Chang et al. 2003; Lohmann et al. 2003). Although it has been proposed that gap junctions which are specialised intercellular junctions be considered as mediators of the PEMF-related cellular responses (Tabrah et al. 1990; McLeod and Rubin 1992; Vander Molen et al. 2000; Lohmann et al. 2003), the underlying mechanism at cellular level that regulates bone remodelling under PEMF remains poorly understood because of the inconsistent or even contradictory results from experiments. For example, cell proliferation, as assayed by cell number and H-thymidine incorporation, has been reported to increase (Monica De et al. 1999), decrease (Fredericks et al. 2000) and remain unaffected (Diniz et al. 2002) by PEMF exposure. Similarly, the production of alkaline phosphatase has been reported to either increase (McLeod and Collazo 2000) or decrease (Vander Molen et al. 2000) the following PEMF exposure.

In order to remove the limitations to generalisation with respect to causes and effects of bone remodelling under PEMF, mathematical models are used to provide a dynamic, quantitative and systematic description of the relationships among interacting components of the biological system. Mathematical modelling is a powerful tool for testing and analysing various hypotheses in complex systems that are very difficult (such as time consuming or money consuming) or just impossible to apply in vivo or in vitro. However, relatively few mathematical models have yet been proposed regarding bone remodelling. Kroll (2000) and Rattanakul et al. (2003) proposed a mathematical model accounting for the differential activity of parathyroid hormone (PTH) administration on bone accumulation. Komarova et al. (2003) presented a theoretical model of autocrine and paracrine interactions among osteoblasts and osteoclasts. Komarova (2005) also developed a mathematical model that describes the actions of PTH at a single site of bone remodelling, where osteoblasts and osteoclasts are regulated by local autocrine and paracrine factors. Potter et al. (2005) proposed a mathematical model for PTH receptor (PTH1R) kinetics, focusing on the receptor’s response to PTH dosing to discern bone formation responses from bone resorption. Lemaire et al. (2004) incorporated detailed biological information and a RANK–RANKL–OPG pathway into the remodelling cycle of a model that included the catabolic effect of PTH on bone, but the anabolic effect of PTH was not described. On the basis of the model of Lemaire et al. (2004), Wang et al. (2009) developed a mathematical model that could simulate the anabolic behaviour of bone affected by intermittent administration of PTH, as well as a parametric study of the control mechanisms of bone remodelling under mechanical stimulus (Wang and Qin 2010). Pivonka et al. developed an extended bone cell population model to explore the model structure of cell–cell interactions theoretically (Pivonka et al. 2008), and then investigated

![Figure 1. Illustration of bone cell model with RANK–RANKL–OPG signalling pathway, PTH and dual action of TGF-β. The symbols (+) or (−) beside each factor indicate a stimulatory or inhibitory action by the factor.](image-url)
the role of the RANK–RANKL–OPG system in bone remodelling (Pivonka et al. 2010).

Although many \textit{in vitro} and \textit{in vivo} studies have been performed, the cellular mechanism by which PEMF affects bone remodelling is still elusive. To the authors’ knowledge, no study has been reported about this area using a mathematical model. To clarify the underlying mechanism at cellular level regulating the effect of PEMF on bone remodelling, based on the cell population dynamics model (Pivonka et al. 2008) and our previous work (Wang et al. 2009; Wang and Qin 2010), the computational system biology method was used to develop a better understanding of bone remodelling under PEMF. Computational system biology uses mathematical modelling to integrate experimental data into a system-level model, which enables the various interactions to be efficiently and methodically investigated (Pivonka et al. 2010). A validated model generated using the computational system biology can be used as a tool to reduce ambiguity of causes and effects in complex systems like bone remodelling, and makes it possible to test various experimental and theoretical hypotheses \textit{‘in silico’} (Defranoux et al. 2005), and subsequently to develop pharmaceutical and clinical interventions for metabolic bone diseases.

2. Model development

2.1 Effects of PEMF on bone remodelling

The assumption that a coupling mechanism must exist between bone formation and resorption was first articulated in 1963 (Frost 1963). However, it took more than 30 years for the exact molecular mechanism describing the interaction between cells of the osteoblastic and osteoclastic lineages to be identified (Anderson et al. 1997). Recent breakthroughs in our understanding of osteoclast differentiation and activation have come from the analysis of a family of biologically related tumour necrosis factor (TNF) receptor (TNFR)/TNF-like proteins: osteoprotegerin (OPG), the receptor activator of nuclear factor (NF)-κB (RANK) and RANK ligand (RANKL), which together regulate osteoclast function (Khosla 2001; Robling et al. 2006). With the discovery of RANK–RANKL–OPG, a revolutionary understanding of osteoclastogenesis was born.

RANK–RANKL–OPG also plays an important role in the local regulation of bone cell function. The overexpression of opposite phenotypes of OPG, as well as RANKL deletion (osteopetrosis), and OPG deficiency or RANKL overexpression (osteoporosis) has led to the hypothesis that OPG and RANKL could be the mediators for the stimulatory or inhibitory effects of a variety of systemic hormones, growth factors and cytokines on osteoclastogenesis (Hadjidakis and Androulakis 2006). This has recently been referred to as ‘the convergence hypothesis’, in that the activities of the resorptive and anti-resorptive agents ‘converge’ at the level of these two mediators, whose final ratio controls the degree of osteoclast differentiation, activation and apoptosis (Hofbauer et al. 2000). PEMF applies its effects on bone cells partly through this pathway, and this concept is supported by a number of studies. In an \textit{in vitro} study (Chang et al. 2004), a PEMF with a frequency of 15 Hz [1 G (0.1 mT); electric field strength 2 mV/cm] was applied to neonatal mouse calcarial bone cell cultures for 14 days. The results demonstrated that PEMF stimulation significantly increased the osteoblasts’ proliferation, and the OPG expression was up-regulated and the RANKL concentration was down-regulated compared to the control group. In another study (Chang et al. 2005), researchers investigated the effects of PEMF with parameters modified from those of clinical bone stimulator devices and concluded that OPG might be an intermediary mediator in the interplay between PEMF stimulation and osteoclastogenesis, where appropriate PEMF intensities could either promote or suppress OPG expressions in osteoblastic lineage. Also, the osteogenetic effect of PEMF was accompanied by a decrease of RANKL. Recent research has also demonstrated that PEMF induces cells in the osteoblast lineage to express OPG (Schwartz et al. 2009).

Several studies have shown that PEMF also causes osteoblasts to produce other paracrine factors, including TGF-β1, prostaglandin E2 (PGE2) and bone morphogenetic protein-2 (Bodamyali et al. 1998; Lohmann et al. 2000; Guerkov et al. 2001). Moreover, macrophage colony-stimulating factor (M-CSF) has been shown to decrease after PEMF exposure (Chang et al. 2005), and bone morphogenetic proteins-2, -4, -5 were found to increase in osteoblasts after PEMF application (Nagai and Ota 1994; Yajima et al. 1996). However, as these observations are not consistent in the literature, these factors are not included in the effects of PEMF on bone remodelling in our model.

PEMF stimulation, unlike drug administration, can produce a local concentration of growth factors synthesis without any systemic side effects. However, it is still important to keep in mind that, as with a drug, the dosage of physical stimulus is fundamental if positive effects on osteogenesis are to be produced. The biological effects of PEMF stimulation depend not only on the length of treatment time but also on the signal characteristics such as intensity, waveform, frequency and length of the signal (Massari et al. 2009). Research (Zhang et al. 2007) has shown that PEMF is more responsive than other waveforms such as rectangular electromagnetic fields (REMF), triangular electromagnetic fields (TEMF) and sinusoidal electromagnetic fields (SEMF) in terms of its effects on the proliferation and differentiation of osteoblastic cells. With regard to the different types of PEMF, Bassett et al. (1981) proposed that single pulse
was superior to burst pulsed PEMF stimulation for osteoporosis prevention and non-union fracture healing, whereas burst pulsed PEMF stimulation had better effects on bone fracture healing acceleration. The study by Hannay et al. (2005) examined the response of osteoblast-like cells to a PEMF stimulus, mimicking that of a clinically available device, using four protocols of the timing of the stimulus, each conducted over 3 days. Protocol 1 stimulated the cells for 8 h each day, protocol 2 stimulated the cells for 24 h on the first day, protocol 3 stimulated the cells for 24 h on the second day and protocol 4 stimulated the cells for 24 h on the third day. In terms of proliferation and differentiation of the cells compared with the control group, no clear trend was observed between the four protocols. Intensity of the PEMF is also an important factor, as demonstrated in data from Chang et al. (2005) which showed that PEMF with different intensities could regulate osteoclastogenesis, bone resorption, OPG, RANKL and M-CSF concentrations in a marrow culture system. In that experiment, the authors used three different electric field intensities of PEMF field (4.8, 8.7 and 12.2 \( \mu \text{V/cm} \)) and observed that the recruitment of osteoclast-like cells was inhibited by approximately 33% and increased by approximately 10% when electric field intensities of PEMF were 4.8 and 12.2 \( \mu \text{V/cm} \), respectively, whereas no significant differences at all time points were observed in the control group.

### 2.2 Mathematical model

Figure 2 presents a schematic diagram of the mathematical model structure of bone remodelling under PEMF.

In the cell population dynamics model, we include three cell populations (see osteoblastic and osteoclastic lineages in Figure 1) into the model equations, namely osteoblastic precursors (OBP), OBA and active osteoclasts (OCA). Uncommitted osteoblastic progenitors (OBU) and osteoclastic precursors (OCP) function as reservoirs, where the cells differentiate into functional cells such as osteoblasts and osteoclasts, respectively, and their numbers are much greater than the functional cells OBP, OBA or OCA. As a result, OBU and OCP are assigned a very large constant compared with other cell numbers in the model (i.e. OBU = OCP = \( 1 \times 10^{-2} \text{pM} \)).

As in Pivonka et al. (2008) and our previous work (Yanan et al. 2009; Wang and Qin 2010), the Hill equation is used to describe the activation and repression of the receptor–ligand interactions. In biochemistry, the Hill equation is used to describe the fraction of the macromolecule saturated by a ligand as a function of the ligand concentration; it is used in determining the degree of cooperativity of the ligand binding to the enzyme or receptor. The equation was originally formulated by Hill (1910) to describe the sigmoidal \( \text{O}_2 \) binding curve of haemoglobin

\[
\theta = \frac{L^n}{K_d + L^n} = \frac{L^n}{K_A^n + L^n},
\]

where \( \theta \) is the fraction of ligand binding sites filled, \( L \) is the ligand concentration, \( K_d \) is the apparent dissociation constant derived from the law of mass action, \( K_A \) is the ligand concentration producing half occupation and \( n \) is the Hill coefficient.

In cell biology, cell responses such as differentiation, proliferation and apoptosis are all related to various ligand–receptor reactions, of which some are stimulatory and others are inhibitory (Pivonka et al. 2008). In the modelling of cell responses, the Hill equation is often used to describe the molecular input function. The activation (act for short) and repression (rep for short) forms of the Hill equation (Alon 2007) for the production rate of a new cell or molecule are (Pivonka et al. 2008)

\[
f'(x^*) = \beta \Pi_{\text{act}} = \frac{\beta x^*}{K_1 + x^*},
\]

\[
f'(x^*) = \beta \Pi_{\text{rep}} = \frac{\beta}{(1 + (x^*/K_2))},
\]

Figure 2. Schematic diagram of the mathematical model structure of PEMF-stimulated bone remodelling at cellular level.
where $x^*$ is the active form of concentration $x$ which is a ligand that governs the production of a cell or molecule $z$ through binding to its receptor on cell, $\beta$ is the maximal production rate of $z$, $K_1$ and $K_2$ are the activation and repression coefficient, $\Pi_{\text{act}}$ and $\Pi_{\text{rep}}$ are activation and repression function of the Hill equation, respectively. Note here that we have already assumed that the Hill coefficient equals one.

For convenience, here and later in the paper, we use the abbreviated forms for the factors involved in the corresponding formulation. As in Figure 1, we used OBU for uncommitted osteoblastic progenitors, OBP for osteoblastic precursors, OBA for mature osteoblast, OCP for osteoclast precursor and OCA for active osteoclasts; we also use RL for RANKL, RK for RANK, Tß for TGF-ß, whereas OPG and PTH remain unchanged.

The equations governing the evolution of the number of osteoblastic and osteoclastic cells at each maturation stage are simply balance equations (Lemaire et al. 2004), which means that each cell stage is fed by an entering flow and is emptied by the outgoing flow of differentiated or apoptotic cells (Figure 1). For example, the incoming flow of the OBP compartment (Figure 1) is differentiated into OBU under the positive effect of TGF-ß, using the activation form of the Hill equation (2); the number of the incoming flow is calculated as $D_{\text{OBP}}\cdot \text{OBP} = \Pi_{\text{act}}^{\text{OBP}}$, where $D_{\text{OBP}}$ is the differentiation rate of preosteoclasts, $\Pi_{\text{act}}^{\text{OBP}}$ is the activation function of TGF-ß’s positive effect on the differentiation of OBU into OBP. The outgoing flow of the OBP compartment is further differentiated into OBA under the negative effect of TGF-ß, using the repression form of Hill Equation (3); the number of this outgoing flow is expressed as $D_{\text{OBP}}\cdot \text{OBP} = \Pi_{\text{rep}}^{\text{OBP}}$, where $D_{\text{OBP}}$ is the differentiation rate of OBP, and $\Pi_{\text{rep}}^{\text{OBP}}$ is the repression function of TGF-ß’s negative effect on the differentiation of OBP into OBA. Consequently, using the balance equation on the OBP compartment, the evolution of the number of OBP is expressed in terms of the difference of the incoming and outgoing flow, which can be seen in the form of Equation (4). The same derivation can be found in Equations (5) and (6). It is assumed in this paper that the bone formation is proportional to the number of active OBA (OBA(t) - OBA(t0)), and the bone resorption is proportional to the number of active OCA (OCA(t) - OCA(t0)), here we start the simulation from the so-called steady state, where BV is 100%, $\text{dBV}/\text{dt} = 0$, correspondingly, OBA(t) is OBA(t0) and OCA(t) is OCA(t0), so the bone volume can be expressed in the form of Equation (7), where BV stands for bone volume in percentage (%), $k_{\text{in}}$ and $k_{\text{out}}$ are the relative bone formation and bone resorption rates, respectively. From Figure 2, it can be seen that the effect of electrical field on bone remodelling is mediated by gap junction and facilitated by the promotion of the secretion of OPG and inhibition of production of RANKL, which can be seen from function $\Pi_{\text{act},\text{OCP}}^{\text{RL}}$ in Equation (6). As a result, utilising Figures 1 and 2 and based on the formulation in Pivonka et al. (2008), we can formulate the bone cell population dynamics as follows:

$$\frac{d\text{OBP}}{dt} = D_{\text{OBP}}\cdot \text{OBP} - \Pi_{\text{act}}^{\text{OBP}},$$

$$\frac{d\text{OBA}}{dt} = D_{\text{OBP}}\cdot \text{OBP} - A_{\text{OBA}}\cdot \text{OBA},$$

$$\frac{d\text{OCA}}{dt} = D_{\text{OCA}}\cdot \text{OCA} - A_{\text{OCA}}\cdot \text{OCA},$$

$$\frac{\text{dBV}}{\text{dt}} = k_{\text{in}}\cdot \text{OBA}(t) - k_{\text{out}}\cdot \text{OCA}(t).$$

where subscript ‘cell’ in the input functions $\Pi_{\text{molecule}}$ means the cell type to which a specific molecule binds and ‘molecule’ denotes the ligand involved in a particular cell response. $D_{\text{OBP}}$ is the differentiation rate of preosteoclasts, $A_{\text{OBA}}$ is the rate of elimination of OBA and $A_{\text{OCA}}$ is the rate of elimination of OCA. All the constants and their values can be found in Appendix B.

Two different timescales are presented in the model: a short timescale is used to describe receptor–ligand reactions such as RANK–RANKL, OPG–RANKL and TGF-ß with its receptor; a long timescale is required to capture the cell number changes such as OBP, OBA and OCA. Note that the receptor–ligand reaction is much faster than the changes in cell numbers, and therefore a quasi-steady-state assumption is used in the model to describe the receptor–ligand reactions.

Bone matrix is the largest source of TGF-ß in the body (Roodman 1999). TGF-ß and growth factors and specific components embedded in the bone matrix are released by osteoclasts during bone resorption (Bonewald and Dallas 1994). The effect of TGF-ß on osteoblasts is bidirectional, depending upon the state of maturation of the osteoblasts (Lemaire et al. 2004). On one hand, TGF-ß has the potential to stimulate osteoblast recruitment, migration and proliferation of osteoblast precursors (meaning OBPs in our model; Bonewald and Dallas 1994). On the other hand, TGF-ß inhibits terminal osteoblastic differentiation into OBAs (Alliston et al. 2001). In this model, we assume that the release rate of TGF-ß from the bone matrix is constant. Using the short timescale and a quasi-steady-state assumption, TGF-ß is (Pivonka et al. 2008)
calculated as
\[ T_B = \frac{\alpha K_{res, OCA} + S_{TB}}{D_{TB}}, \] (8)

where \( S_{TB} \) is a source/sink term for TGF-\( \beta \), \( \alpha \) is the TGF-\( \beta \) content stored in bone matrix, \( K_{res} \) is the relative rate of bone resorption, \( D_{TB} \) is the rate of degradation of TGF-\( \beta \). Consequently, the activation and repression forms of TGF-\( \beta \) can be obtained by substituting Equation (8) into Equations (2) and (3):

\[ \Pi^T_{act,OBU} = \frac{T_B}{K_{D1,TB} + T_B}, \] (9)

\[ \Pi^T_{rep,OBP} = \frac{1}{1 + T_B/K_{D2,TB}}, \] (10)

\[ \Pi^T_{act,OCA} = \frac{T_B}{K_{D3,TB} + T_B}, \] (11)

where \( K_{D1,TB} \) is the activation coefficient related to TGF-\( \beta \) binding on OBU, \( K_{D2,TB} \) is the repression coefficient related to TGF-\( \beta \) binding on OBP and \( K_{D3,TB} \) is the activation coefficient of TGF-\( \beta \) binding on OCA. Applying the law of mass action (Lemaire et al. 2004) used to describe the reactions of receptors and corresponding ligands, the formulations including PTH with its receptor, RANKL with OPG and RANKL with RANK can be found in our previous work (Wang et al. 2009).

In the model, we take PTH as a regulator of RANKL and OPG production. The assumptions are made that PTH endogenous production is constant and PTH_max > PTH. There is an equal degree of PTH binding to its receptors on OBP and OBA, to obtain PTH concentration and its according activation and repression functions as (Pivonka et al. 2008):

\[ PTH = \frac{\beta_{PTH} + P_{PTH,act}(t)}{D_{PTH}}, \] (12)

\[ \Pi^T_{act,OBP} = \frac{PTH}{K_{D4,PTH} + PTH}, \] (13)

\[ \Pi^T_{act,OPG} = \frac{1}{1 + PTH/K_{D5,PTH}}, \] (14)

where \( \beta_{PTH} \) is the synthesis rate of systemic PTH, \( P_{PTH,act}(t) \) represents an external PTH dosing term, \( D_{PTH} \) is the rate of degradation of PTH. \( K_{D4,PTH} \) is the activation coefficient for RANKL_eff on OBP related to PTH binding and \( K_{D5,PTH} \) is the repression coefficient for OPG production related to PTH binding on OBA.

As stated above, we already have concluded on the basis of experimental observations that PEMF stimulates the production of OPG expressed in OBA and inhibits the expression of RANKL in OBP (see Section 2.1), and OPG and RANKL are the only two factors that are considered to be regulated by PEMF stimulation and modelled in the model. The systemic hormone PTH down-regulates OPG production in OBA (Wang et al. 2009). Therefore, based on Pivonka et al. (2008), the OPG concentration can be expressed as

\[ OPG = \frac{\beta_{OPG,OBP}(TPH_{rep,OBP} + F_{OPG}(t))}{\beta_{OPG,OBP}(TPH_{rep,OBP} + F_{OPG}(t)) + OPG_{max}} + D_{OPG}, \] (15)

where \( \beta_{OPG} \) is the production rate of OPG per OBA, \( F_{OPG}(t) \) is the influence of PEMF on OPG secretion characterised by its intensity, frequency, time and waveform, \( P_{OPG,act}(t) \) is an external OPG administration term, \( D_{OPG} \) is the rate of degradation of OPG and \( OPG_{max} \) is the maximum possible OPG concentration. Also, PEMF inhibits RANKL expression in OBP and PTH up-regulates the RANKL ‘effective carrying capacity’ of OBP (Wang et al. 2009). Building on the idea of Pivonka et al. (2008), we can obtain the concentration of RANKL as follows:

\[ RL = \left( \frac{R_{RL,OBP} \Pi^T_{PTH,act,OBP}}{1 + K_{A1,RL,OPG} \cdot K_{A2,RL,RK}} \right) \times \left( \frac{\beta_{RL,OBP} \cdot F_{RL}(t) + P_{RL,act}(t)}{\beta_{RL,OBP} \cdot F_{RL}(t) + D_{RL} \cdot R_{RL,OBP} \cdot \Pi^T_{PTH,act,OBP}} \right), \] (16)

where \( R_{RL} \) is the maximum RANKL on OBP, \( K_{A1,RL} \) is the association binding constant RANKL–OPG, \( K_{A2,RL} \) is the association binding constant RANKL–RANK, \( \beta_{RL} \) is the production rate of RANKL per OBP, \( F_{RL}(t) \) is the effect of PEMF on RANKL production characterised by its intensity, frequency, time and waveform, \( P_{RL,act}(t) \) is an external RANKL administration term and \( D_{RL} \) is the rate of degradation of RANKL. Then the activation function of RANKL on differentiation of osteoclast precursor cells OCP can be obtained using Equations (2) and (16),

\[ \Pi^T_{act,CLP} = \frac{RL}{K_{D6,RL} + RL}, \] (17)

where \( K_{D6,RL} \) is the activation coefficient related to RANKL binding on OCP.

3. Numerical investigation and parametric study of the model
3.1 Numerical investigation
Bone remodelling is an important biological system when it comes to fracture bone healing, fracture non-union and bone diseases like osteoporosis. It is mediated executed by the coordinated activities of osteoclastic cells and osteoblastic cells. At present, bone remodelling is modelled using systems of partial differential equations (PDEs) with the main focus on the growth and differentiation of cells.
cells in BMUs. The coupling between osteoclastic cells and osteoblastic cells is facilitated by the RANK–RANKL–OPG pathway, together with the systemic hormone PTH and TGF-β. PEMF devices are used clinically to promote bone healing, especially in fracture non-union, but relatively little is known about the mechanisms involved. In this paper, we have proposed a mathematical model to simulate the effects of PEMF on bone remodelling at cellular level, which can lead to better understand the underlying mechanisms. In this section, we have solved the ordinary differential Equations (4)–(7) numerically using Matlab and plotted a series of graphs about the concentration dynamics of OPG, RANKL, cell populations of OBA, OCA and OBP and bone volume, as presented in Figures 3–6, respectively. Note that the parameter values are from the models on which this model is based (Pivonka et al. 2008) and from our previous work (Yanan et al. 2009; Wang and Qin 2010). The effects of PEMF on bone remodelling are characterised by its intensity, frequency, waveform, application time, etc. and according to study of Hannay et al. (2005), the timing of PEMF stimulation does not affect bone cell development, which is different from the bone remodelling that occurs under mechanical stimulus. In this numerical investigation, the specific parameter values of PEMF were adopted from widely used clinical PEMF devices (Li et al. 2007), and this set of parameter values is the only one used in our model. Consequently, we assume that the effects of PEMF on bone remodelling, specifically OPG or RANKL in our model, do not change (represented by two different constants $F_{OPG}$ and $F_{RL}$ in the model) all the way through the simulation (3 months).

The concentration dynamics of OPG during 3 months of PEMF application are simulated in Figure 3. Consistent with experimental observations, the OPG concentration increases during the first 10 days of simulation. Surprisingly, the concentration of OPG in simulation decreases continually from the 10th day. Regrettably, because most in vitro experiments have been done over 2 weeks, there is no available experimental data to compare with. The pattern of the OPG concentration might be explained as follows: because of the effect of the PEMF stimulus, OPG concentration rises over a short period after its application; then the binding of OPG with RANKL catches up as bone remodelling occurs, more OPG are consumed than produced.

![Figure 3. OPG concentration dynamics during 3-month PEMF application.](image)

![Figure 4. RANKL concentration dynamics during 3-month PEMF application.](image)

![Figure 5. OBA, OCA and OBP cell population dynamics during 3-month PEMF application.](image)
by osteoblast cells, and, as a result, OPG concentration decreases until the end of the simulation.

Figure 4 shows the RANKL concentration dynamics during the 3-month PEMF application. As can be seen from the graph, within 2 weeks (which is the timescale of most in vitro experiments), the RANKL concentration drops, compared with the initial value. However, it was not anticipated that the RANKL concentration would increase significantly immediately after the simulation began, followed by a dramatic decrease, and then remain at same level through the rest of the simulation. A possible reason for this pattern is that the number of preosteoblast cells that produce RANKL is increased by the stimulus effect of the PEMF on the proliferation of bone marrow mesenchymal stem cells (Sun et al. 2009) immediately after its application, then the inhibitory effect of the PEMF on the OBP dominates, and this overall trend persists until the end of the simulation.

The cell population dynamics of OBA, OCA and OBP are shown in Figure 5. As expected, the OBA and OBP populations increase and OCA decreases in the first 2 weeks of the simulation, which is consistent with experimental observations. Because of the coupling effect between OCA and OBA, the OBA population starts to drop after it reaches its peak and continues to decrease until the end of simulation while maintaining a higher concentration than OCA, which accounts for the continuing growth of bone volume in Figure 6.

3.2 Parametric study of control mechanism of bone remodelling under PEMF

In this section, based on the mathematical model developed above, we perform an extensive parametric study investigating the model parameters related to fundamental cell behaviours such as differentiation and apoptosis, in order to identify putative control mechanisms for physiologically reasonable bone remodelling under PEMF.

The functional outputs of the bone remodelling system, such as bone loss or gain or homeostasis, are executed by BMUs, where osteoclasts absorb bone mineral in bone matrix and activated osteoblasts lay down the newly formed bone. The BMU acts as a mediator mechanism bridging individual cellular activity to whole bone morphology (Frost 1986), which is sensitive to any changes in its microenvironment. As a result, it is expected that any modification to the BMU component will have a significant effect on its output behaviour.

From a control theory point of view, one can always argue that there must be several control mechanisms working simultaneously in the complex bone remodelling system under PEMF, governing the response of the BMU to changes in its microenvironment by modifying the differentiation or apoptosis rates of bone cells. In this paper, we apply perturbations to the bone remodelling system under PEMF (which is in steady state) by down- and up-regulating its parameters in random combination groups of five differentiation and apoptosis rate parameters $D_{OFBU}$, $D_{OCF}$, $D_{OBP}$, $A_{OBA}$ and $A_{OCA}$. In this case, each parameter in each group (group of one, two, then three, four and finally all five parameters at a time) can up- or down-regulate, and using simple combination theory, we can calculate that the total number of permutations is $242 = \sum_{i=1}^{5} C_{5}^{i} 2^{i}$. Then, in order to investigate the system behaviour for a wide range of changes, we apply an exponentially changed factor which is $1.5^x$ to each of the five differentiation and apoptosis rate parameters, where the exponent ex ranges from $-10$ to $10$ in step increases of $0.5$. The assessment of the effects of each of the parameter combinations on the system behaviour is chosen as the responses of bone volume which are sampled on 90th day to represent the maximum change. Using Matlab, we can plot all the 242 graphs. Then, summarising all the plots of bone volume vs. variation of exponent ex, we find that there are three subsets of curves, which are plotted in Figure 7.

Figure 7(a), (b) shows an exponential increase and decrease of bone volume, respectively, when the model parameter is increased exponentially (exponent $ex$ from $-10$ to $10$). This type of behaviour is considered physiologically unrealistic from a biological viewpoint, although it was obtained for quite a large range of model parameter combinations. On the other hand, Figure 7(c) represents the other extreme case, where only minor changes of bone volume occur over the entire range of parameter variation. These three types of response curves were excluded from our further analysis on the grounds...
that they did not provide an effective control mechanism for bone volume.

In Pivonka et al. (2008), the 'idealised' regulatory response by functionally active BMUs is discussed. As we stated earlier, the bone remodelling system is initiated from a steady state, where we can identify $D_{BV} = 0$, and concentrations of various hormones and growth factors cause initial values of differentiation and apoptosis rates in BMUs. In order to respond to minor tiny changes in concentrations, it is expected that BMUs should be insensitive to these fluctuations. Therefore, from Figure 8, we can recognise point A as the threshold concentration, which means that any change of model parameter below A causes no change in bone volume. Also, a region around the usual operation status of BMUs should be found with relatively small gradients of change in bone volume in

![Figure 7](image1.png)  
**Figure 7.** Physiologically, unrealistic changes of BMC and BFE vs. combined changes of model parameter $[1.5^{-10} - 1.5^{+10}] \cdot p$, where $p$ is the parameter value: (a) exponential bone growth, (b) exponential bone decrease and (c) slight changes of bone.

![Figure 8](image2.png)  
**Figure 8.** Schematic illustration of ideal response curve for combined changes of model parameters.
response to changes in differentiation rates (regions C–D and D–E in Figure 8) and larger gradients for larger changes in differentiation rates (region E–F in Figure 8). Although it is expected that the response in bone volume change will remain limited, the differentiation rates increase significantly (region beyond point F in Figure 8), because the bone volume increases unlimitedly and is not physiologically realistic. On the other hand, it is expected that the rate of bone volume change will also decrease to a limited extent if the differentiation rates decrease significantly. As a matter of fact, physiologically it is reasonable for the bone volume change to be zero for extremely small differentiation rates. Additionally, it can be seen that point F in Figure 8 marks the maximum change in bone volume (ΔB max). Since we have point A, which is the maximum concentration that does not lead to further modifications of bone volume, there must be a transition region from point C to point A, which is characterised by point B, the lowest value of bone volume.

After having found a potential ‘ideal response curve’, we can now begin to search for response curves that might meet these requirements. Encouragingly, we have been able to identify a small number of curves that display similarity to the idealised response curve.

Table 1 summarises all the parameter combinations that produce idealised response curves. In Figure 9, we plot the physiologically realistic response curve that corresponds to the parameter permutation involving three parameters (A_{OBA}, A_{OCA}, A_{OST} = −/ +/) and is similar to the idealised response curve shown in Figure 8.

4. Conclusions

In this paper, a mathematical model of bone remodelling under PEMF at cellular level was proposed based on experimental results and our previous work. This model incorporates the latest experimental findings through extensive literature review and summarises that PEMF applies its physiological effects via RANK–RANKL–OPG pathway, specifically that PEMF promotes the secretion of OPG and inhibits the production of RANKL, which overall suppresses the population of osteoclasts that absorb bone minerals. Using this model as a basis, the numerical investigation demonstrated the concentration dynamics of growth factors OPG and cytokines RANKL and the population dynamics of OBA, OBP and OCA. More importantly, parametric study of bone remodelling under PEMF was carried out in order to understand the control mechanism of bone remodelling at cellular level under PEMF. From a control mechanism perspective, it is quite likely that there are several control mechanisms working simultaneously in bone remodelling, which is a complex system. Consequently, we performed an extensive

---

Table 1. Summary of parameter combinations that lead to controlled remodelling process.

<table>
<thead>
<tr>
<th>Number of parameters in a combination</th>
<th>Combinations of differentiation and apoptosis rates</th>
<th>Variation of each parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>D_{OBB}/A_{OBA}</td>
<td>+/+</td>
</tr>
<tr>
<td>2</td>
<td>D_{OCP}/A_{OST}</td>
<td>+/−</td>
</tr>
<tr>
<td>2</td>
<td>A_{OBA}/A_{OCA}</td>
<td>−/+</td>
</tr>
<tr>
<td>3</td>
<td>D_{OBB}/A_{OBA}/A_{OCA}</td>
<td>−/+/−</td>
</tr>
<tr>
<td>3</td>
<td>D_{OBB}/A_{OBA}/A_{OCA}</td>
<td>−/+/−</td>
</tr>
<tr>
<td>3</td>
<td>D_{OBB}/A_{OBA}/A_{OST}</td>
<td>+/+/+</td>
</tr>
<tr>
<td>3</td>
<td>A_{OBA}/A_{OCA}/A_{OST}</td>
<td>+/+/+/</td>
</tr>
<tr>
<td>4</td>
<td>D_{OBB}/A_{OBB}/A_{OBA}/A_{OCA}</td>
<td>−/+/−/−/−</td>
</tr>
<tr>
<td>4</td>
<td>D_{OBB}/A_{OBB}/A_{OBA}/A_{OCA}</td>
<td>−/+/−/−/−</td>
</tr>
<tr>
<td>4</td>
<td>D_{OBB}/A_{OBB}/A_{OBA}/A_{OST}</td>
<td>−/+/−/−/−</td>
</tr>
<tr>
<td>4</td>
<td>A_{OBA}/A_{OCA}/A_{OST}</td>
<td>−/+/−/−/−</td>
</tr>
<tr>
<td>5</td>
<td>D_{OBB}/A_{OBB}/D_{OCP}/A_{OBA}/A_{OST}</td>
<td>+/+−/+</td>
</tr>
<tr>
<td>5</td>
<td>D_{OBB}/A_{OBB}/D_{OCP}/A_{OBA}/A_{OST}</td>
<td>−/+−/+</td>
</tr>
<tr>
<td>5</td>
<td>D_{OBB}/D_{OBB}/D_{OCP}/A_{OBA}/A_{OST}</td>
<td>−/+−/+</td>
</tr>
<tr>
<td>5</td>
<td>A_{OBA}/A_{OCA}/A_{OST}</td>
<td>−/+−/+</td>
</tr>
</tbody>
</table>

Note: The variation with ‘+’ represents parameter increase, and ‘−’ represents parameter decrease.

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Figure 9. Typical physiologically realistic fluctuations of bone volume with combinations of parameter change.
parametric study investigating the model parameter space related to cell differentiation and apoptosis, which described the fundamental cell lineage behaviours, to investigate such a scenario. After analysing all the combinations (that is 242 permutations) of five model parameters, we successfully identified a small number of parameter combinations that could cause physiologically realistic responses which were similar to the theoretically idealised physiological response. This work furthers our understanding of bone remodelling under PEMF. The control mechanisms identified will be helpful in the development of combined pharmacological and PEMF therapies to treat bone loss diseases like osteoporosis.

Acknowledgements
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References


Appendix A

Initial values of the model variables

\[ 0 = D_{OBU} \Pi_{act,OBU}^{Tb} - D_{OBP} \Pi_{rep,OBP}^{Tb}, \]  
\[ 0 = D_{OBP} \Pi_{act,OBP}^{Tb} - A_{OBA} \cdot OBA, \]  
\[ 0 = D_{OCA} \Pi_{act,OCA}^{Tb} - A_{OCA} \cdot OCA \cdot \Pi_{rep,OCA}^{Tb}. \]  

Solving Equations (A1)–(A3), we obtain the initial values of the model variables as follows:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>OBA (0)</td>
<td>pM</td>
<td>0.2126991130e-3</td>
</tr>
<tr>
<td>OBP (0)</td>
<td>pM</td>
<td>0.8986869185e-5</td>
</tr>
<tr>
<td>OCA (0)</td>
<td>pM</td>
<td>0.2769166993e-3</td>
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Appendix B

Parameter values and descriptions (parameter values are from Pivonka et al. 2008 and Lemaire et al. 2004) except where otherwise indicated.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_{OBU})</td>
<td>pM/day</td>
<td>(7 \times 10^{-4})</td>
<td>Differentiation rate of uncommitted OB progenitors</td>
</tr>
<tr>
<td>(D_{OBP})</td>
<td>pM/day</td>
<td>5.348</td>
<td>Differentiation rate of preosteoblasts</td>
</tr>
<tr>
<td>(D_{OCP})</td>
<td>pM/day</td>
<td>(2.1 \times 10^{-3})</td>
<td>Differentiation rate of preosteoclasts</td>
</tr>
<tr>
<td>(A_{OBA})</td>
<td>pM/day</td>
<td>(1.890 \times 10^{-1})</td>
<td>Rate of elimination of OBA</td>
</tr>
<tr>
<td>(A_{OCA})</td>
<td>pM/day</td>
<td>(7.000 \times 10^{-1})</td>
<td>Rate of elimination of OCA</td>
</tr>
<tr>
<td>(K_{D1,Tb})</td>
<td>pM</td>
<td>(4.545 \times 10^{-3})</td>
<td>Activation coefficient related to TGF-(b) binding on OBU</td>
</tr>
<tr>
<td>(K_{D2,Tb})</td>
<td>pM</td>
<td>(1.416 \times 10^{-3})</td>
<td>Repression coefficient related to TGF-(b) binding on OBP</td>
</tr>
<tr>
<td>(K_{D3,Tb})</td>
<td>pM</td>
<td>(4.545 \times 10^{-3})</td>
<td>Activation coefficient of TGF-(b) binding on OCA</td>
</tr>
<tr>
<td>(K_{D4,PTH})</td>
<td>pM</td>
<td>(1.500 \times 10^{2})</td>
<td>Activation coefficient for RANKL\textsuperscript{eff} on OBP related to PTH binding</td>
</tr>
<tr>
<td>(K_{D5,PTH})</td>
<td>pM</td>
<td>(2.226 \times 10^{2})</td>
<td>Repression coefficient for OPG production related to PTH binding on OBA</td>
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<tr>
<td>(K_{D6,RL})</td>
<td>pM</td>
<td>(1.500 \times 10^{2})</td>
<td>Activation coefficient related to RANKL binding on OCP</td>
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<tr>
<td>(R_{RL})</td>
<td>pM</td>
<td>(1 \times 10^{1})</td>
<td>Unchanged concentration of RANK</td>
</tr>
<tr>
<td>(R_{OPG})</td>
<td>pM/cell</td>
<td>(3 \times 10^{9})</td>
<td>Maximum RANKL on OBP</td>
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<tr>
<td>(\beta_{PTH})</td>
<td>pM/cell</td>
<td>(2.5 \times 10^{2})</td>
<td>Synthesis rate of systemic PTH</td>
</tr>
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<td>(\beta_{RL})</td>
<td>pM/cell</td>
<td>(1.684 \times 10^{4})</td>
<td>Production rate of RANKL per OBP</td>
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<tr>
<td>(\beta_{OPG})</td>
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<td>(1.464 \times 10^{6})</td>
<td>Production rate of OPG per OBA</td>
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<td>(D_{PTH})</td>
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<td>Rate of degradation of PTH</td>
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<tr>
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<td>Coefficient of PEMF’s effect on OPG (Chang et al. 2004, 2005; Schwartz et al. 2009)</td>
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<tr>
<td>(D_{Tb})</td>
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<td>Rate of degradation of TGF-(b)</td>
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<tr>
<td>(k_{Tb})</td>
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<td>0.5</td>
<td>Relative influence of TGF-(b) binding in OBU differentiation</td>
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<tr>
<td>(k_{PTH})</td>
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<td>0.7</td>
<td>Relative influence of PTH binding in production of OPG in OBA</td>
</tr>
<tr>
<td>(K_{A1,RL})</td>
<td>pM\textsuperscript{-1}</td>
<td>(1 \times 10^{-3})</td>
<td>Association binding constant RANKL–OPG</td>
</tr>
<tr>
<td>(K_{A2,RL})</td>
<td>pM\textsuperscript{-1}</td>
<td>(3.412 \times 10^{-2})</td>
<td>Association binding constant RANKL–RANK</td>
</tr>
<tr>
<td>(OPG_{max})</td>
<td>pM</td>
<td>(2 \times 10^{8})</td>
<td>Maximum possible OPG concentration</td>
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<tr>
<td>(\alpha)</td>
<td>%</td>
<td>1</td>
<td>TGF-(b) content stored in bone matrix</td>
</tr>
<tr>
<td>(k_{res})</td>
<td>Day\textsuperscript{-1}</td>
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<td>Relative rate of bone resorption</td>
</tr>
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<td>Relative rate of bone formation</td>
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