The role of impairment of mesenchymal stem cell function in osteoporotic bone fracture healing

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ABSTRACT

With demographic change and increasing life expectancy, osteoporotic fractures have become one of the most prevalent trauma conditions seen in daily clinical practice. A variety of factors are known to affect the rate of healing in osteoporotic conditions (*e.g.* both biochemical and biomechanical environment of callus cells). However, the influence of impairment of mesenchymal stem cell function in the osteoporotic condition on bone fracture healing has not been fully understood. In the present study, we develop a mathematical model that quantifies the change in biological processes within the fracture callus as a result of osteoporosis. The model includes special features of osteoporosis such as reduction in mesenchymal stem cell (MSC) number in osteoporotic bone, impaired response of osteoporotic MSCs to their biomechanical microenvironment and the effects of configuration of locking compression plate (LCP) system on healing in this context. The results presented here suggest that mechanical-mediated MSC differentiation at early stages of healing are significantly affected under osteoporotic conditions, while it is predicted that the flexible fixation achieved by increasing bone-plate distance of LCP could alleviate the negative effects of osteoporosis on healing. The outcomes of this study could potentially lead to patient specific surgical solutions, and thus achieve optimal healing outcomes in osteoporotic conditions.

Key words: Mesenchymal stem cells; osteoporosis; bone fracture healing; mechano-regulation; locking compression plate.

INTRODUCTION

As the world's population is ageing rapidly, osteoporosis becomes a highly prevalent and costly disease which is predicted to affect 1,555 million worldwide by 2050 [1]. The most frequent fractures among elderly patients are associated with osteoporosis [2]. Compared with normal fracture healing, osteoporotic fracture healing of the femoral diaphysis is significantly delayed by around three weeks [3]. In addition, biomechanical evaluation of osteoporotic healing using a sheep model also demonstrated a delay of fracture healing in osteoporotic bones with a significant reduction in torsional and bending stiffness by 33% and 21%, respectively, in comparison to normal healing[4].

The ability of biological tissues to repair themselves following trauma depends on the biological actions of mesenchymal stem cells (MSCs), which are gradually diminished with increasing age [5]. Immediately after a fracture, MSCs migrate into the fracture site, where they exert their biological actions by responding to their biochemical and biomechanical microenvironment [6]. However, clinical studies have shown that the repair capacity of ageing bone progressively declines as a result of a deficit of bone MSCs [7]. Further, MSCs from osteoporotic patients feature impaired signal transduction which could have a negative impact on callus formation, endochondral ossification and bone morphogenetic protein (BMP) mediated MSC migration and invasion, ultimately a delayed healing [8,9].

It is well known that mechano-regulation plays an important role in the course of bone healing [10-13], especially at the early stage of healing [14]. Recent studies generally agree that the octahedral shear strain and interstitial fluid velocity are two mechanical stimuli that modulate callus cell proliferation and differentiation by triggering a cellular response [15]. However, bone cells could have an impaired response to their mechanical microenvironment [16] due to relatively fewer mesenchymal stem cells and impaired proliferative response in

osteoporotic bone [17,18].

The investigation of the effects of the stress-driven flow of interstitial fluid on bone cells showed that, after one hour of stress-driven flow of interstitial fluid, the mechanical stimuli mediated upregulation of prostaglandin E_2 and TGF- β is significantly reduced in osteoporotic patients in comparison to normal subjects [16]. Using a series of theoretical approaches in conjunction with mechanical testing, our recent studies have suggested that the healing outcomes could be enhanced by increasing interfragmentary (IFM) movement through making locking compression plate (LCP) system more flexible by adjusting the bone-plate distance and working length of LCP [19-24]. Therefore, it is reasonable to speculate that the fixation design of LCP can be strategically modified to achieve improved fracture healing outcomes in osteoporotic bone.

Despite conclusive evidence that there is delayed and/or impaired fracture healing in osteoporotic bone, the influence of osteoporosis on fracture healing has not yet been fully understood and clinical evidence is still lacking. As shown in Fig 1.a, the purpose of this study is to develop a computational model to investigate the impaired fracture healing resulting from the deficient response of MSCs in osteoporotic bone and explore the effective ways of improving the healing outcomes by strategically modifying the configuration of LCP.

METHODS

While the detailed mechanisms of age-related osteoporotic bone fracture healing are far from clear to date, we make the following assumptions for simplicity and without sacrificing the fundamental behaviours exhibited by the complicated healing processes:

- Immediately after fracture, MSCs are released into the callus tissue from the periosteum, surrounding soft tissues and bone marrow[25].

- The differentiation rates of MSCs into osteoblasts, chondrocytes and fibroblasts are mainly regulated by their biomechanical microenvironment of the cells [25], while the effects of growth factors (*e.g.* chondrogenic growth factor and osteogenic growth factor) [26] are ignored in this study.
- The impact of osteoporosis is mainly on the reduction of MSCs number in bone and impaired response of MSCs to their mechanical stimuli [16-18].
- The major source of fibroblasts, chondrocytes and osteoblasts in the callus is mainly from the differentiation of MSCs.
- The degradation and proliferation of MSCs, fibroblasts, chondrocytes and osteoblasts in callus are ignored in this present study.

Below, we present the modelling approach and formulations employed in this study.

Formulations

The methodology proposed in present study is based on the theory of porous media to model the mechanical behaviour of soft fracture callus at early stage of healing [21,22,19,23,13,27]. The callus is treated as a triphasic mixture consisting of (1) a solid phase representing extracellular matrix (ECM) of callus; (2) a fluid phase representing interstitial fluid; and (3) a solute phase consisting of callus cells (*i.e.* MSCs, chondrocytes, osteoblasts and fibroblasts) which migrate within the callus. As the volume of solute phase is relatively small compared to solid and fluid phases, we may assume

$$\phi^s + \phi^f = 1 \quad (1)$$

where ϕ^s and ϕ^f are volume fraction of solid and fluid phase, respectively.

Transport of MSCs (c^m)

The transport of MSCs within the callus can be expressed as:

$$\frac{d(\phi^f c^m)}{dt} = -\nabla \bullet (-\phi^f D_m \nabla c^m + \phi^f \mathbf{v}^f c^m) - \phi^f (s_d^{fb} + s_d^c + s_d^b) c^m - k_d^m \quad (2)$$

where D_m is diffusion coefficient of MSCs and $\mathbf{v}^f$ is the true fluid velocity related to a fixed representative element volume (REV). s_d^{fb} , s_d^c and s_d^b are the differentiation rates of MSCs into fibroblasts, chondrocytes and osteoblasts, respectively. k_d^m is the degradation rate of MSCs, and $\nabla \bullet$ denotes the divergence operator.

Transport of fibroblasts (c^{fb})

$$\frac{d(\phi^f c^{fb})}{dt} = -\nabla \bullet (-\phi^f D_{fb} \nabla c^{fb} + \phi^f \mathbf{v}^f c^{fb}) + \phi^f s_d^{fb} c^m - s_d^{fb} \quad (3)$$

where D_{fb} is diffusion coefficient of fibroblasts, and s_d^{fb} the degradation rate of fibroblasts.

Transport of chondrocytes (c^c)

$$\frac{d(\phi^f c^c)}{dt} = -\nabla \bullet (-\phi^f D_c \nabla c^c + \phi^f \mathbf{v}^f c^c) + \phi^f s_d^c c^m - s_d^c \quad (4)$$

where D_c is diffusion coefficient of chondrocytes, and s_d^c the degradation rate of chondrocytes.

Transport of osteoblasts (c^b)

$$\frac{d(\phi^f c^b)}{dt} = -\nabla \bullet (-\phi^f D_b \nabla c^b + \phi^f \mathbf{v}^f c^b) + \phi^f s_d^b c^m - s_d^b \quad (5)$$

where D_b is diffusion coefficient of osteoblasts, and s_d^b the degradation rate of osteoblasts.

Modelling mechanical behaviour of soft fracture callus at early stage of healing

Traditionally, the mechanical behaviour of soft tissues such as callus is modelled using the theory of porous media with considering infinitesimal strain theory in the formulation and

applying a linear elastic constitutive relation for modelling the solid phase. However, the soft fracture callus at early stage of healing can undergo very large deformation under physiologically relevant loading (*i.e.* up to 100% without rupture) [28], and therefore application of infinitesimal strain theory cannot accurately capture the mechanical behaviour of soft callus. In this study the large-deformation mechanical behaviour of early fracture callus is modelled by formulating the classical theory of porous media in Lagrangian framework.

Assuming the early stage of fracture callus is homogeneous and undergoing large deformation, the mechanical quantities (*e.g.* interstitial fluid velocity, $\mathbf{v}^f$) can be determined through the conservation of linear momentum [29], that is

$$\nabla \bullet (-p\mathbf{I} + \sigma_E^s) = 0 \quad (6)$$

where σ_E^s is effective stress of solid phase which can be modelled by using the incompressible Neo-Hookean material model which is widely used in describing the large deformation behaviour of biological tissues (*e.g.* soft callus) [30,29], that is

$$\sigma_E^s = \frac{1}{J^s} \mathbf{F}^s \bullet 2 \frac{\partial(\mathbf{W}^s)}{\partial \mathbf{C}^s} \bullet \mathbf{F}^{s^T} \quad (7a)$$

$$\mathbf{W}^s = C_1(\bar{I}_1 - 3) + D_1(J^s - 1)^2 \quad (7b)$$

$$C_1 = \mu_0 / 2 \quad (7c)$$

$$D_1 = K_0 / 2 \quad (7d)$$

where J^s is the Jacobian determinant of the deformation gradient of the solid phase [31], $\mathbf{F}^s$ the deformation gradient of the solid phase and $\mathbf{C}^s$ the right Cauchy-Green deformation tensor of the solid phase. $\mathbf{W}^s$ is the energy density function of incompressible Neo-Hookean model, $\bar{I}_1$ the first invariant of the isochoric part of the right Cauchy-Green deformation

163 tensor, μ_0 the initial shear modulus, and K_0 the initial bulk modulus.

164 The governing equation which links the calls ECM deformation to the interstitial fluid
 165 motion can be obtained through conservation of mass of solid and fluid phase with
 166 consideration of the solid reference configuration to be the “true” reference configuration and
 167 redefined all fluid quantities with respect to the solid skeleton [31], that is

$$168 \quad \dot{\mathbf{W}} + \mathbf{k} \bullet \nabla_0 p = 0 \quad (8a)$$

$$169 \quad \dot{\mathbf{W}} = J^s \mathbf{F}^{s-1} \bullet [\phi^f (\mathbf{v}^f - \mathbf{v}^s)] \quad (8b)$$

$$170 \quad \mathbf{k} = J^s \mathbf{F}^{s-1} \bullet \boldsymbol{\kappa} \bullet \mathbf{F}^{s-t} \quad (8c)$$

171 where $\dot{\mathbf{W}}$ is the Lagrangian fluid velocity relative to the solid phase, ∇_0 the material
 172 gradient operator on solid phase, and $\boldsymbol{\kappa}$ the symmetric Darcy permeability tensor.

173 *Mechano-regulation*

174 Experimental evidence suggest that the MSCs response to mechanical stimuli exhibits a
 175 threshold behaviour, with different differentiation behaviours triggered when the value of
 176 “mechanical stimulation index (S)” reaching different thresholds [32], *i.e.* (1) osteoblast
 177 differentiation when $S < 1$; (2) chondrocyte differentiation when $1 < S < 3$; and (3) fibroblast
 178 differentiation when $S > 1$. The value of mechanical stimulation index (S) is determined by
 179 both octahedral shear strain of the solid phase (τ^s) and the interstitial fluid velocity relative to
 180 solid phase (v^f , $\mu m/s$) [32], that is

$$181 \quad S = k_d^s \times \left(\frac{\tau^s}{a} + \frac{v^f}{b} \right) \quad (9)$$

182 where $a = 0.0375$ and $b = 3\mu m/s$ [32]. k_d^s is the reduction factor of MSCs response to
 183 mechanical stimuli ($0 \leq k_d^s \leq 1$) due to the impact of osteoporosis.

Model geometry

As shown in Fig 1.b, a fractured human tibia with a 3 mm fracture gap size supported by a locking compression plate (LCP) was modelled in this study. It was assumed that the fracture is supported by a 4.5 mm broad DePuy Synthes stainless steel LCP. The plates were 206 mm long, 17.5mm wide and 5.2 mm thick with 11 locking holes. The 3D geometries of tibia and LCP were created by using our previously developed techniques [22,21] involving CT scan images in conjunction with commercial medical imaging processing software Mimics (Materialise, Belgium) and CAD software Solidworks.

Loading protocol

The fractured bone was subject to a quasi-static compressive load (*i.e.* 150N in total, equivalent to approximately 20% of the normal body weight, which is suitable for a patient after operation [21]) uniformly distributed on the top of the cortical bone.

Boundary conditions

It is assumed that the exterior boundary of early stage fracture callus is impermeable (*i.e.* no flux) [33]. Further, it is assumed that the majority of MSCs are from the exterior boundary of periosteal callus and bone marrow [25], that is

$$\bar{c}_0^m = k_d^m c_0^m \quad (10)$$

where c_0^m is MSCs concentration at exterior boundary of periosteal callus and bone marrow under normal condition. k_d^m is reduction factor ($0 \leq k_d^m \leq 1$) due to the impact of osteoporosis.

Initial conditions

It is assumed that initially there is no cell in fracture callus, that is

$$c^m(t=0)=0, c^c(t=0)=0, c^b(t=0)=0, c^{fb}(t=0)=0 \quad (11)$$

Numerical considerations

The governing equations (2)-(8) were solved numerically by using commercial finite element (FE) package COMSOL MULTIPHYSICS [34]. The soft fracture callus was meshed with 14,335 second-order tetrahedral elements (Fig 1.b), which were determined based on convergence study with an error tolerance of 2%. The values of parameters used in this simulation are listed in Table 1.

The present study compared the numerical predictions of three scenarios (*i.e.* Cases 1-3 in Table 1) representing the impact of osteoporosis on the number of MSCs in bone ($k_d^m = 0.5$, where k_d^m is the reduction factor of MSCs number in bone as shown in Equation 10), the impaired response of MSCs to their biomechanical microenvironment ($k_d^s = 0.5$, where k_d^s is the reduction factor of MSCs response to mechanical stimuli as shown in Equation 9) under different LCP configurations (*i.e.* bone-plate distance = 2~4mm) relative to Control (*i.e.* normal condition: $k_d^m = 1$, $k_d^s = 1$ and bone-plate distance = 2mm).

RESULTS

Fig 2 shows the distribution of the mechanical simulation index (S) across the fracture callus (Section A-A) with consideration of the impact of osteoporosis and different bone-plate distance ranging from 2mm to 4mm. It can be seen from Fig 2b (Case 1) that, compared to normal condition (Control), the mechanical simulation index (S) is significantly reduced due to the impaired response of MSCs. However, with the increase of the flexibility of LCP (*i.e.* increasing bone-plate distance from 2mm to 4mm), the impact of osteoporosis on mechanical stimuli can be alleviated as shown in Fig 2c-d.

The role of the flexibility of LCP in alleviating the impaired healing resulting from

osteoporosis was further investigated by simulating the change in callus MSCs differentiation to osteoblasts, chondroblasts and fibroblasts under different LCP configurations (*i.e.* bone-plate distances) (Fig 3). It can be seen that, in comparison to Case 1 (*i.e.* bone-plate distance = 2mm), there is little change in osteoblast, chondroblast and fibroblast concentration in callus by increasing bone-plate distance from 2mm to 3mm (Case 2). However, when the bone-plate distance is increased to 4mm (Case 3), the impact of osteoporosis could significantly be alleviated. For example, compared to Case 1, a bone-plate distance of 4mm is capable of alleviating the impact of osteoporosis on osteoblasts and chondroblasts concentration by 21% and 30%, respectively, but there is little change in fibroblast concentration. The implication of these results is that a relatively flexible fixation could potentially encourage endochondral ossification and the development of fracture calls in osteoporotic condition which is favourable for fracture healing [35].

DISCUSSION

The current model predictions are consistent with the growing evidence that indicates, at early stage of healing, the loading regime required for osteoporotic bone cell simulation is different from that in normal condition [36,16]. It generally agrees that the mechanical stimuli mediated bone fracture healing is governed by the movement (IFM) of fracture site [37,38]. It is believed that the stiffness of an osteoporotic fracture fixation construct has to be increased as osteoporosis decreases the mechanical properties of bone. However, an overly stiff fixation construct may lead to insufficient IFM at the fracture site which further inhibits the mechanical stimuli mediated healing in osteoporotic condition, resulting ultimately in delayed healing or non-union [39]. While IFM of the fracture site depends on the physiological activities of a patient after surgery, excessive loading could result in complications (*e.g.* increasing the risk of failure of implant fixation) due to the reduced

mechanical quality of osteoporotic bone. The present study demonstrates that the application of locking compression plate (LCP) system provides a way of enhancing IFM without increasing the magnitude of the loading (Fig 3) due to the fact that clinically the flexibility of LCP could be enhanced by adjusting the working length, bone-plate and number of screws [40]. The implication of this research is that fixation design can be strategically modified based on the predictions from theoretical models to achieve improved fracture healing outcomes in osteoporotic patients.

At early stage of healing, it is reasonable to assume that the majority of MSCs in callus is from the periosteum, surrounding soft tissues and bone marrow [25]. Further, the current study mainly focused on investigating the effects of reduced MSCs number in osteoporotic bone and the impaired mechano-regulation of osteoporotic bone cells on bone healing progression. However, the effects of osteoporosis on other biological factors that influence the healing outcomes were not taken into consideration in this present study. For example, recent studies found that osteoporosis significantly affects the expression of vascular endothelial growth factor (VEGF) [41], which plays an important role in the formation of new vasculature in callus (*i.e.* angiogenesis), and so delivers necessary oxygen and nutrients for endochondral ossification [42]. The impaired angiogenesis in osteoporotic condition may also lead to delayed healing or non-union, and therefore requires further investigation.

CONCLUSIONS

In this present study, we developed a computational model to investigate the impact of impairment of MSCs induced by osteoporosis on bone fracture healing and explore the ways of modifying the flexibility of LCP to improve the healing outcomes in osteoporotic bone. A summary of the main results are as follows:

- The osteoporosis induced decrease in number of MSCs in osteoporotic bone and the

impaired response of MSCs to their mechanical stimuli could significantly reduce MSCs differentiation to osteoblast and chondroblast at early of healing (*e.g.* a 50% reduction factor may lead to 55% and 47% decrease in osteoblast and chondroblast concentration in early stage soft callus, respectively).

- By using a more flexible LCP through increasing bone-plate distance, the effects of osteoporosis on healing could be significantly alleviated. For example, by increasing the bone-plate distance from 2mm to 4mm, the comparison of the simulation results from Case 1 and Case 3 shows that the serious impact of osteoporosis on osteoblast and chondroblast concentration in callus can be reduced by 21% and 30%, respectively.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table Caption

Table 1 Parameters used throughout this study [43,11,44-47,25].

Parametric study			
Control	BPD = 2mm, $k_d^s = k_d^m = 1.0$		
	BPD - LCP Bone-plate distance;		
	k_d^s - Reduction factor of the sensitivity of MSCs to mechanical stimuli;		
	k_d^m - Reduction factor of MSC concentration at boundary		
Case 1	BPD = 2mm, $k_d^s = k_d^m = 0.5$		
Case 2	BPD = 3mm, $k_d^s = k_d^m = 0.5$		
Case 3	BPD = 4mm, $k_d^s = k_d^m = 0.5$		
Material properties of bone			
	Porosity	Poisson's Ratio	Permeability (m ⁴ /Ns)
Granulation Tissue	0.8	0.167	10 ⁻¹⁴
Marrow	0.8	0.167	10 ⁻¹⁴
Cortical bone	0.04	0.3	10 ⁻¹⁷
Material properties of stainless LCP			
Young's modulus (GPa)	220		
Poisson's ratio	0.34		
Material properties of fracture callus for Neo-Hookean material model			
Initial shear modulus (μ_0)	0.021		
Initial bulk modulus (K_0)	0.025		
Mesenchymal stem cells, chondrocytes, osteoblasts and fibroblasts			
Diffusion coefficient of mesenchymal stem cells (D_m)	9.765×10^{-13} m ² /s		
Diffusion coefficient of chondrocytes (D_c)	1×10^{-30} m ² /s		
Diffusion coefficient of osteoblasts (D_b)	1×10^{-30} m ² /s		
Diffusion coefficient of fibroblasts (D_{fb})	2.84×10^{-12} m ² /s		
Rate of mechanical stimuli mediated chondrocyte differentiation (s_d^c)	0.3/day		
Rate of mechanical stimuli mediated osteoblast differentiation (s_d^b)	(0.005 + 0.145 × S)/day, where S is mechanical simulation index		
Rate of mechanical stimuli mediated fibroblast differentiation (s_d^{fb})	0.01/day		

Figure Caption

Fig 1 The proposed modelling scheme. (a) Scope of this study incorporating impact of osteoporosis on healing outcomes; (b) A schematic diagram of a fractured tibia bone supported by a locking compression plate (LCP) system undergoing physiological relevant

loading. The flexibility of LCP can be adjusted by working length (WL) and bone-plate distance (BPD) of the LCP.

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Fig 2 The distribution of reduced mechanical simulation index (S) in the fracture callus (Section A-A) with consideration of the impact of osteoporosis.

Fig 3 Percent change in differentiation of chondrocytes, osteoblasts and fibroblasts under different cases (i.e. Cases 1-3, Table 1) compared to control respectively.