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Oxygen as a critical determinant of bone fracture healing—A multiscale model

Aurélie Carlier [a,b,d], Liesbet Geris [a,b,d], Nick van Gastel [c,d], Geert Carmeliet [c,d], Hans Van Oosterwyck [a,d,*]

[a] Biomechanics Section, KU Leuven, Celestijnenlaan 300 C, PB 2419, 3000 Leuven, Belgium
[b] Biomechanics Research Unit, University of Liege, Chemin des Chevreuils 1—BAT 52/3, 4000 Liege 1, Belgium
[c] Clinical and Experimental Endocrinology, KU Leuven, O&N 1, Herestraat 49, PB 902, 3000 Leuven, Belgium
[d] Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, O&N 1, Herestraat 49, PB 813, 3000 Leuven, Belgium

HIGHLIGHTS

- The influence of oxygen was incorporated in a multiscale model of fracture healing.
- The results of the oxygen model were compared with experimental observations.
- An extensive sensitivity analysis of the oxygen model indicated its robustness.
- Adequate spatiotemporal oxygen patterns appear to be critical for bone healing.

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ABSTRACT

A timely restoration of the ruptured blood vessel network in order to deliver oxygen and nutrients to the fracture zone is crucial for successful bone healing. Indeed, oxygen plays a key role in the aerobic metabolism of cells, in the activity of a myriad of enzymes as well as in the regulation of several (angiogenic) genes. In this paper, a previously developed model of bone fracture healing is further improved with a detailed description of the influence of oxygen on various cellular processes that occur during bone fracture healing. Oxygen ranges of the cell-specific oxygen-dependent processes were established based on the state-of-the art experimental knowledge through a rigorous literature study. The newly developed oxygen model is compared with previously published experimental and *in silico* results. An extensive sensitivity analysis was also performed on the newly introduced oxygen thresholds, indicating the robustness of the oxygen model. Finally, the oxygen model was applied to the challenging clinical case of a critical sized defect (3 mm) where it predicted the formation of a fracture non-union. Further model analyses showed that the harsh hypoxic conditions in the central region of the callus resulted in cell death and disrupted bone healing thereby indicating the importance of a timely vascularization for the successful healing of a large bone defect. In conclusion, this work demonstrates that the oxygen model is a powerful tool to further unravel the complex spatiotemporal interplay of oxygen delivery, diffusion and consumption with the several healing steps, each occurring at distinct, optimal oxygen tensions during the bone repair process.

1. Introduction

1.1. Normal fracture healing

When a bone fractures, the bone architecture distorts and blood vessels rupture, thereby filling the fracture site with blood which rapidly coagulates to form a blood clot or hematoma (Murao et al., 2013). Since the damaged vasculature fails to provide sufficient oxygen and nutrients, the injury site gradually becomes hypoxic and the surrounding tissues start to degrade (Cameron et al., 2013). This triggers the invasion of inflammatory cells, macrophages and leukocytes, marking the start of the inflammatory phase. Simultaneously, growth factors and cytokines produced by the cells in the hematoma and surrounding tissues attract fibroblasts, mesenchymal stem cells (MSCs) and endothelial cells to the trauma site (Taguchi et al., 2005). The fracture callus fills with granulation tissue, forming the soft callus, in which the MSCs start

* Corresponding author at: Biomechanics Section, KU Leuven, Celestijnenlaan 300 C, PB 2419, 3000 Leuven, Belgium. Tel.: +32 16 327067; fax: +32 16 327994.
E-mail address: hans.vanoosterwyck@mech.kuleuven.be (H.V. Oosterwyck).

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to differentiate. In the periosteal region, near the bone cortex where oxygen is available, the MSCs differentiate directly towards osteogenic cells. These newly formed osteoblasts produce a woven bone matrix (intramembranous ossification). In the hypoxic central fracture area, the mesenchymal stem cells will first differentiate into chondrocytes which produce a cartilaginous template that mechanically stabilizes the fracture zone. The hard callus formation stage starts with the invasion of blood vessels into this cartilaginous template. The new-sprung vasculature brings along osteoblasts that produce a hard tissue callus of mineralized woven bone matrix (endochondral bone formation). When the bony callus bridges the fracture gap, a clinical union is reached. In the final remodeling phase, the hard callus is remodeled by osteoclasts and osteoblasts, gradually replacing the immature woven bone by lamellar bone and returning the bone to its original shape, size and strength (Einhorn, 1998).

1.2. The role of oxygen in fracture healing

Oxygen is essential for multiple cellular functions occurring during normal conditions as well as during repair processes like fracture healing. First, it is required for the aerobic metabolism of cells, thereby producing ATP for normal cellular function (Lu et al., 2013a; Maes et al., 2012). Second, oxygen is important for the activity of many enzymes (Lu et al., 2013a; Xie et al., 2009). Third, a lack of oxygen induces the expression of several (angiogenic) genes through the hypoxia inducible factor (HIF)-pathway (Pugh and Ratcliffe, 2003;Maes et al., 2012; Wan et al., 2008; Komatsu and Hadjiargyrou, 2004; Bouletreau et al., 2002). Fourth, through the molecular mechanisms mentioned above, oxygen has a profound effect on the differentiation and proliferation capacity of MSCs, chondrocytes and osteoblasts (Malladi et al., 2006; Xu et al., 2007; Grayson et al., 2007; Lennon et al., 2001; Holzwarth et al., 2010; Wagegg et al., 2012; Zscharnack et al., 2009; Meyer et al., 2010; Hirao et al., 2006; Ren et al., 2006; Merceron et al., 2010). Lennon et al. (2001) observed for example that rat MSCs proliferated faster and formed more colonies in low oxygen (5% oxygen tension) than in control conditions (20% oxygen tension). Similar results were obtained by Grayson et al. (2007). They report a 30-fold higher expansion of the human MSCs under 2% oxygen tension with respect to 20% oxygen tension (Grayson et al., 2007). Finally, it was shown that prolonged hypoxic conditions lead to cell death, delayed chondrogenic and osteogenic differentiation and impaired fracture healing (Lu et al., 2007, 2013b; Brinker and Bailey, 1997).

1.3. Mathematical models of fracture healing including oxygen

Since oxygen influences many critical processes of fracture healing, as was mentioned in the previous section, mathematical models of fracture healing should consider it explicitly, in this way providing additional opportunities to deepen the scientific understanding of the biological mechanisms at hand. In this section we will give a brief overview of the most recent mathematical models of fracture healing that include oxygen, as this is the focus of this study. For comprehensive reviews on mathematical models of fracture healing, we refer the reader to Geris et al. (2009), Isaksson (2012) and Pivonka and Dunstan (2012).

Simon et al. used fuzzy logic rules to describe the interaction between mechanical stability, revascularization and tissue differentiation during fracture healing with three main variables: vascular perfusion, cartilage concentration and bone concentration (Simon et al., 2011; Chen et al., 2009). They show that both mechanical stabilization as well as sufficient nutrient supply are essential for bone healing since a less stabilized osteotomy leads to slower revascularization and delayed bony bridging. An inadequate nutrient supply, resulting from an increased gap size, would also lead to the formation of a non-union. However, they model the dynamics of endothelial cells as well as the nutrient delivery by diffusion equations with constant diffusion coefficients, i.e. the vessel growth will continue until a uniform density is reached. As such, Chen et al. (2009) fail to capture the prolonged absence of healing resulting in a clinical non-union since their model, given enough time, will eventually result in complete bony bridging.

Burke and Kelly, (2012) were able to predict all the major events of fracture repair by defining substrate stiffness and oxygen tension as key regulators of MSC differentiation. However, they model angiogenesis as a diffusive process, thereby neglecting the discrete nature of the vascular tree (Burke and Kelly, 2012). Moreover, in their model only the differentiation of MSCs is made oxygen dependent (Burke and Kelly, 2012), whereas experimental evidence indicates that multiple cellular processes are regulated by oxygen.

Geris et al. (2008) developed a model that describes the bone regeneration process as a spatiotemporal variation in density of 12 continuous variables: mesenchymal stem cells, chondrocytes, osteoblasts, fibroblasts, endothelial cells, cartilage matrix, bone matrix, fibrous matrix, vascular matrix, osteogenic growth factors, chondrogenic growth factors and angiogenic growth factors. Peiffer et al. (2011) extended the fracture healing model developed by Geris et al. (2008) by including a discrete, lattice-free description of endothelial tip cell migration and angiogenesis instead of a continuum description of the vasculature (by means of a vascular density). This modification not only resulted in a more realistic description of angiogenesis, it also allowed to explicitly model oxygen as a variable influencing the fracture healing process through its release from the newly formed vessel network. As such, the model of Peiffer et al. correctly captured the different aspects of bone regeneration as well as some important aspects of angiogenesis like blood vessel growth, branching and anastomosis (Peiffer et al., 2011). The model of Peiffer et al. (2011) was further refined by Carlier et al. (2012) by introducing an intracellular level in every endothelial cell describing the Dll4-Notch signaling pathway thereby replacing the phenomenological rules of tip cell selection used by Peiffer et al. (2011). Due to its multiscale nature, the so called MOSAIC model (multiscale model of osteogenesis and sprouting angiogenesis with lateral inhibition of endothelical cells) of Carlier et al. (2012) was able to simulate the bone regeneration process accurately, as well as to reproduce many experimentally observed aspects of tip cell selection: the salt and pepper pattern seen for endothelial cell fates, an increased tip cell density in heterozygous Dll4 knockout cases and an excessive number of tip cells in high VEGF (vascular endothelial growth factor) environments.

1.4. Objectives of this study

As indicated above, oxygen clearly plays a key role in fracture healing. Indeed, it appears that many biological processes that take place during fracture healing (e.g. proliferation, differentiation, cell death) occur at cell-specific optimal oxygen tensions. Some of the most recent mathematical models of fracture healing have tried to incorporate the role of oxygen in fracture healing, however none of the aforementioned models (including our own) explicitly captures the influence of oxygen on cellular proliferation, differentiation, hypoxia signaling and cell death. We hypothesize that the spatiotemporal distribution of oxygen tension, influenced by amongst others cellular consumption as well as the timely revascularization of the callus, is an important determinant of fracture healing. Therefore, this study will establish a new computational model of fracture healing that is able to more accurately describe the regulatory properties of oxygen on cellular processes occurring during normal and impaired fracture healing. This goal is accomplished by combining the state-of-the-art knowledge on