A multiscale model of sprouting angiogenesis during fracture healing

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Introduction

The healing of a fracture strongly depends on the development of a new blood vessel network (angiogenesis) in the fracture zone. The current computational models of angiogenesis represent the process of tip cell selection either at a large scale with phenomenological rules [Peiffer *et al*, 2011; Qutub *et al*, 2009] or at a small scale using detailed agent-based models [Bentley *et al*, 2008]. This study presents a novel multiscale model of osteogenesis and sprouting angiogenesis, incorporating lateral inhibition of endothelial cells (further denoted MOSAIC model) through Dll4-Notch1 signaling, and applies it to fracture healing.

Materials and Methods

The model integrates an intracellular module, which represents the Dll4-Notch1 pathway that determines tip cell selection [Bentley *et al*, 2008], into the hybrid model of Peiffer *et al* [2011]. Fig. 1 gives a schematic overview of the MOSAIC model which consists of:

(1) *a tissue level* describing the various key processes of bone regeneration with 11 continuous variables: mesenchymal stem cells (MSCs), fibroblasts (FBs), chondrocytes (CHs), osteoblasts (OBs), fibrous matrix (m_f), cartilage (m_c), bone (m_b), oxygen (n) and osteogenic (g_b), chondrogenic (g_c) and vascular (g_v) growth factors,

(2) *a cellular level* representing the developing vasculature with discrete endothelial cells (ECs)

(3) *an intracellular level* that defines the internal dynamics of every EC (Dll4-Notch1 signaling).

The model was implemented in MATLAB (The MathWorks, Inc).

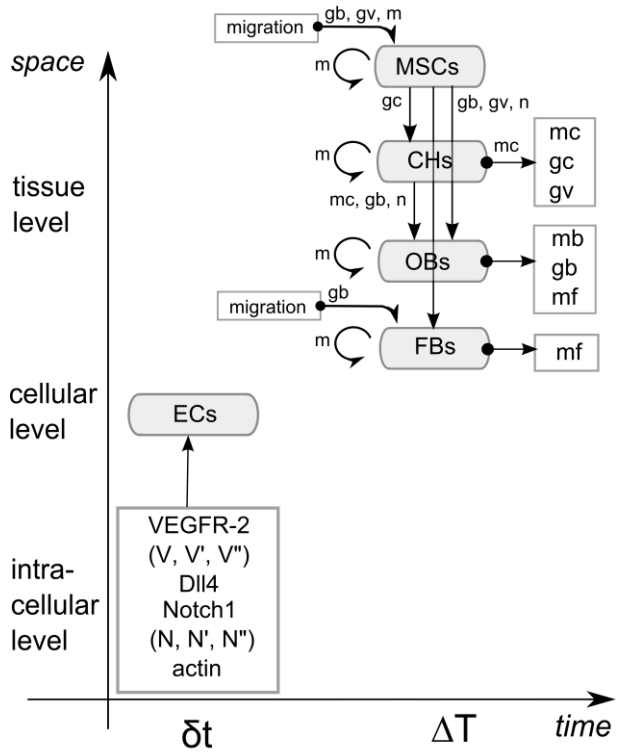

Figure 1. Scale separation map indicating schematically the modeled processes at different spatial and temporal scales. The intracellular variables govern endothelial cell (EC) behavior. Cell types that are considered at the tissue scale (MSCs: mesenchymal stem cells, CHs: chondrocytes, OBs: osteoblasts, FBs: fibroblasts) can migrate (only MSCs and FBs), proliferate (circular arrows), differentiate (vertical arrows) and produce growth factors (*gb*: osteogenic growth factor concentration, *gc*: chondrogenic growth factor concentration, *gv*: angiogenic growth factor concentration) and extracellular matrix (*mf*: fibrous tissue density, *mb*: bone density, *mc*: cartilage density, *m*: total tissue density). Blood vessels serve as an oxygen source (*n*: oxygen concentration). Variables next to an arrow indicate their mediating role for a certain tissue level process.

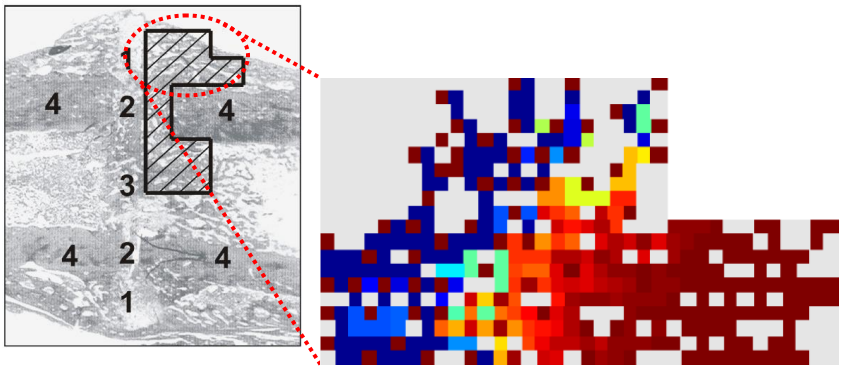

Figure 2. (left) Geometrical domain: 1. periosteal callus, 2. intercortical callus 3. endosteal callus 4. cortical bone (right) Detail of the periosteal callus showing the VEGFR-2 levels in every EC. Remark that the tip cells have high VEGFR-2 levels (brown) and the stalk cells low (blue).

Results and Discussion

The MOSAIC model predicts the evolution of the continuous variables (fig. 3) as well as the evolution of the intracellular variables (fig. 2) during normal fracture healing. Many experimentally observed aspects of tip cell selection are correctly recapitulated by the simulation results, such as: (1) the 'salt and pepper' pattern of cell fates (fig. 2), i.e. a tip cell with high VEGFR-2 and actin levels followed by a stalk cell with correspondingly low levels due to Notch signaling, (2) an increased tip cell density in case of Dll4 inhibition and (3) excessive tip cell numbers in high VEGF concentrations.

Fig. 3 compares the predictions of the Peiffer-model [Peiffer *et al*, 2011] and the MOSAIC model with the experimentally measured tissue fractions of Harrison et al. [2003] in a rodent standardized fracture model. Both models capture the general trends in the experimental data: the bone tissue fraction gradually increases throughout the healing process; the fibrous tissue fraction disappears; the cartilage template is first produced and later replaced by bone. After one, two and three weeks the surface fraction of the blood vessels in the callus is respectively 3.05%, 35.54% and 65.86%.

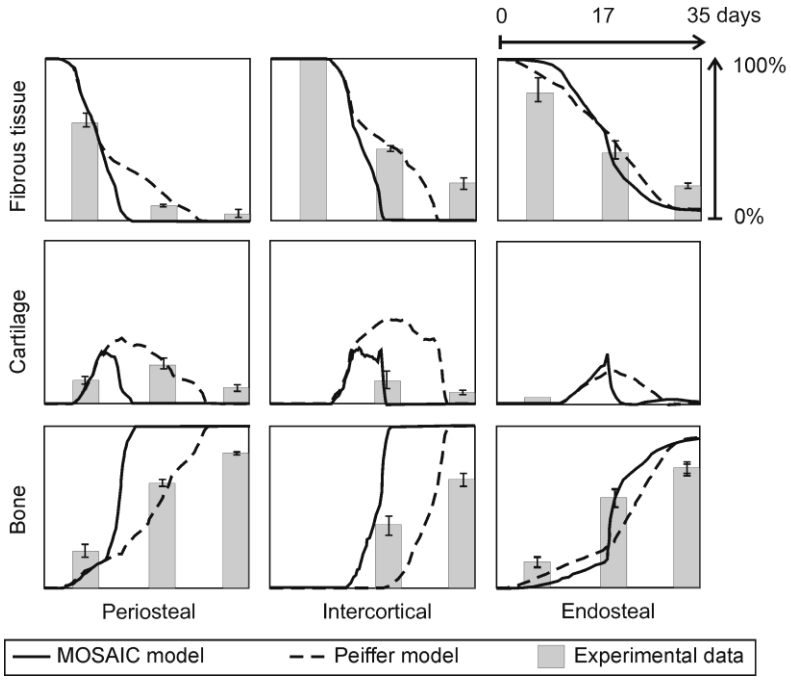

Figure 3. *In silico* and *in vivo* evolution of normal fracture healing. Temporal evolution of the bone, cartilage and fibrous tissue fractions (%) in the periosteal, intercortical and endosteal callus as predicted using the hybrid model of Peiffer et al. [2011] and the newly developed multiscale model (MOSAIC) and as measured by Harrison et al. [2003].

The MOSAIC model can also be used to investigate the influence of high or low VEGF environments on the development of the vasculature and the progression of the healing. The VEGF concentration can be decreased by adding VEGF-antibodies or by increasing the amount VEGFR-1, a decoy receptor for VEGF. The model predicts that an increase of the latter results in a reduction of the vascular density since less VEGF remains available for VEGFR-2 activation (Fig. 4), which is also seen in loss-

and gain-of-function data [Krueger *et al*, 2011]. Consequently, the reduced vascularization will delay the healing of the fracture or even impair it, thereby resulting in a non-union.

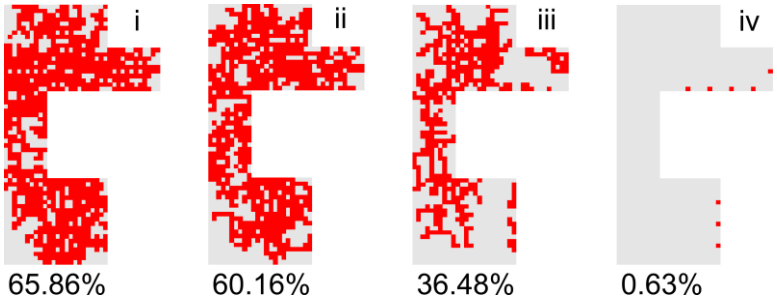

Figure 4. Vasculature at 35 days post fracture for a variation in the amount of decoy receptor VEGFR-1. i: V_{sink} = 0.275 (standard condition), ii: V_{sink} = 0.125, iii: V_{sink} = 0.025, iv: V_{sink} = 0.0025.

Conclusion

This study presents a multiscale model of osteogenesis and sprouting angiogenesis with lateral inhibition of endothelial cells (MOSAIC). The bone regeneration process was predicted by the model in accordance with experimental reports and previously validated *in silico* results [Geris *et al*, 2008]. The MOSAIC model correctly recapitulated many experimentally observed aspects of tip cell selection such as the salt and pepper pattern seen for cell fates and an increased tip cell density due to the loss of Dll4. The proposed model incorporates biological processes at various temporal and spatial scales, a technique with many advantages, some of which have been demonstrated here. Firstly, information at all three scales can be extracted and used to analyze the model: the influence VEGF-antibody injection or blockage of VEGF-receptors on fracture healing can be further investigated with this multiscale framework. Secondly, the intracellular levels are meaningful for experimental biologists, thereby establishing a bridge between computational and experimental biologists. Finally, the proposed multiscale model is more mechanistic since tip cell selection is based on intracellular dynamics, rather than some phenomenological "rules" as was the case in the hybrid model of Peiffer et al. [2011]. This allows gaining more insight into the fundamental processes at the micro-scale that in turn give rise to the emergent behavior seen at the macro-scale. In conclusion, the proposed multiscale model was found to be a useful tool to investigate possible biological mechanisms across different time and spatial scales, thereby contributing to the fundamental knowledge of sprouting angiogenesis in both normal and pathological conditions.

Acknowledgements

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References

Bentley, K., Gerhardt, H., Bates, P.A. 2008 Agent-based simulation of notch-mediated tip cell selection in angiogenic sprout initialisation. *J. Theor. Biol.* 250, 25-36. (doi:10.1016/j.jtbi.2007.09.015)

Geris, L., Gerisch, A., Vander Sloten, J., Weiner, R., Van Oosterwyck, H. 2008 Angiogenesis in bone fracture healing: a bioregulatory model. *J. Theor. Biol.* 251, 137-158. (doi:10.1016/j.jtbi.2007.11.008)

Harrison L.J., Cunningham J.L., Stromberg L. et al. 2003 Controlled induction of a pseudarthrosis: A study using a rodent model. *J. Orthop. Trauma*. 17, 11-21. (doi: 10.1097/00005131-200301000-00003)

Krueger J., Liu D., Scholz K., et al. 2011 Flt1 acts as a negative regulator of tip cell formation and branching morphogenesis in the zebrafish embryo. *Development*. 138, 2111-2120. (doi: 10.1242/dev.063933)

Peiffer, V., Gerisch, A., Vandepitte, D., Van Oosterwyck, H., Geris, L. 2011 A hybrid bioregulatory model of angiogenesis during bone fracture healing. *Biomech Model Mechanobiol.* 10, 383-395. (doi:10.1007/s10237-010-0241-7)

Qutub, A., Popel, A. 2009 Elongation, proliferation and migration differentiate endothelial cell phenotypes and determine capillary sprouting. *BMC Syst. Biol.* 3, 13. (doi:10.1186/1752-0509-3-13)