

INVESTIGATION AND MODELING OF BONE FRACTURE HEALING

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Abstract

An initial stage of bone fracture healing, a clot formation is discussed in this paper. This is an important step in a healing process and it determines future formation of a callus and resulting tissue formation and remodeling. Preliminary results of an animal experiment and theoretical considerations are presented. A biomechanical model of considered phenomenon is proposed and a simple numerical example is discussed. In this example square domain of a porous tissue surrounding broken bone is considered. In this domain micro-cracks are generated. Their distribution changes linearly with a distance from a surface contacting with a bone. The open pores are filled with a physiological liquid. One of the edges of the considered domain is in contact with the fractured bone. It is assumed that blood leaks from the fracture in bone, mixes with the fluids in the pores and propagates into the porous tissue. After a certain time period blood solidification starts what results in a clot formation. It is assumed that the shape of the clot is determined by an assumed level of tissue saturation with blood component at the moment when solidification starts. It follows from this investigations that after minor improvements of the theoretical description and experimental determination of the parameters necessary to perform calculations this model can be used in future to predict conditions necessary for correct and fast bone fracture healing.

Introduction

Problem Formulation

Bone fracture healing is a complex process dependent on biochemical and biomechanical factors. Good mathematical models of this process and related numerical simulations may offer many important informations useful in surgery planning and post-operation treatments. Fracture healing consists of several consecutive phases. The final goal of our work is to model in a consistent way all of the phases and to include in the formulation the effects of mechanical stimulation on the activities of cells involved in bone tissue regeneration. In the present paper selected preliminary results of the theoretical and experimental investigations concerning the first, very important phase of fracture healing are presented.

Biomechanical Aspects of Bone Fracture Healing

Bones have a unique ability to recover after injury and to adapt to variable in time and space biomechanical conditions. There are two scenarios of bone healing discussed in the literature. In the present paper we focus our attention on so-called secondary healing, more important for practical reasons. Bone fracture healing process consists of four important stages namely: 1) hematoma and clot formation; 2) inflammatory phase; 3) reparative phase; 4) remodeling phase, see e.g. (McCollister and Churchill-Livingstone, Ed., 1990; Mandracchia et al., 2001; Gerstenfeld et al., 2003). As an effect of injury the periosteum covering bone surface is often mechanically broken. The same happens with blood vessels close to the fracture. The first two phases are associated with each other and the sharp distinction between them is difficult. During this initial step some important effects occur which determine the future stages of healing process. Mechanical damages result in blood leaking and its propagation into the pores present in the vicinity of a crack. Soon after a contact with the other tissues a solidification of blood starts. This process is crucial in stopping endless bleeding and in building a scaffold necessary for cell activities and future recovery of bone tissue. Solidification of blood results in clot formation. It is composed of fibrin forming a complex 3-D network and connected blood plates, leukocytes and erythrocytes, see e.g. (Twardowska and Kucharczyk, 2009). Complex and important biochemical and biomechanical effects occur after blood vessels damage. They are not discussed here in detail because of space lack. In general, one can say that mechanical damages of vessels and periosteum cause exposition of different tissues to contact with blood and clot factors and secretion of biochemical factors which induce vessels contraction and swelling of soft tissues in the surrounding domain. Initialization of clot formation occurs about 15 second after severe injury and in 1-2 minutes in case of petty wound. It results usually in stopping of bleeding after 3-6 minutes, see e.g. (Stryer, 2003). The next, third step represents the reparative phase. During this stage a formation of so-called callus occurs. Callus represents a kind of bridge connecting the broken elements of bone and enabling mechanical stabilization of bone fragments. Its formation is based on a clot structure

created during earlier phases and endochondral bone formation mechanism is involved in this process, see e.g. (Einhorn, 1998; Yoo and Johnstone, 1998; Felisbino and Carvalho, 2001). The bone remodeling and functional adaptation is characteristic for the final, fourth phase of bone healing. During this phase the resorption of callus takes place and recovery of compact bone occurs. It follows from this short discussion that indeed the formation of clot during the initial stages of bone fracture healing plays a crucial role in a complete process.

Methods

Experimental Observations

An animal experiment has been performed in order to investigate the effects of different bio-mechanical factors on fracture healing process in three groups of sheep model. Three animals have been examined in each group. Each animal was subjected to osteotomy of tibia and external stabilizers were used then to keep 3mm gap between cut bone fragments. The first group was exposed to regular mechanical stimulation using specially designed vibration exciter. In the second group in addition to mechanical stimulation, an injection of PDGF, enriched plasma, was applied into the fracture gap. The last group was the control one. The observations lasted approximately three months. In the present work we focus only on the first phase of healing so the clot formation and following it callus formation undergo examination. In addition to RTG pictures after the 2, 5 and 8 weeks after the surgery the investigation of fractured bones at the end of the experiment using micro-CT and histology examinations are planned.

Modeling of a Clot Formation

In the present section the first phase of bone fracture healing is discussed and an attempt to model the basic phenomena present in hemorrhage and clot formation is made. As mentioned in the previous section the bone fracture healing can be classified as primary or secondary. In this paper we focus on the secondary class which is more important. After bone fracture an inflammation state starts and the blood coming from the ruptured blood vessels causes hemorrhage and clot formation which is a key point in future callus formation and tissue regeneration. Blood coming from damaged blood vessels propagates in the fracture adjacent domain and after contact with surrounding tissues within few minutes forms a clot - a scaffold which serves as a supporting structure for mesenchymal cells coming from periosteum and from marrow stromal cells. These cells in the next stage proliferate and depending on the local mechanical conditions and biological environment differentiate either into osteoblasts bone forming cells, into chondroblasts cartilage specific cells or into fibroblasts characteristic for fibrous tissue. On the basis of formed clot callus is build which primary function is to fix fractured fragments of bone. As it follows from this explanation hemorrhage and following clot formation play a fundamental role in future events necessary for good healing and remodeling of bone. In the present discussion we assume that tissue surrounding the fractured bone is porous with open pores filled with a liquid. Due to inflammation and resulting swelling this is assumed that the permeability of this tissue is not constant in space and decreases with distance from the region where the fracture appeared. The blood coming from broken vessels mixes with the liquid present in the pores and propagates into the porous tissue. In contact with unfamiliar tissue within short time it forms a solid structure which disables further propagation and determines clot formation and its shape. The time necessary to initiate solidification of blood depends on biological factors and together with the severity of fracture and its geometry determines an amount of blood coming out of vessels and the region of porous tissue reached by the blood. In this consideration we assume that after a given, assumed period of time the blood forms a solid structure in the regions where its saturation reaches a given assumed level. This way by doing analysis of flow of mixture blood/surrounding-liquids in a porous media we are able to determine the region where the clot formation is present.

To collect the basic equations let us consider two phase flow in a domain Ω . We assume that the motion of fluids is dominated by viscous effects and the effects of gravity, compressibility and capillary pressure are neglected. Porosity is assumed to be linearly decreasing with distance from the bone surface. We denote variables associated to each of two phases, blood and liquid present in the

pores, using subscripts b and l . According to Darcy's law the velocities of molecules of each of the two phases are proportional to the pressure gradient:

$$\mathbf{u}_i = -\frac{k_{ri}(S)}{\mu_i} \mathbf{K} \cdot \nabla p \quad (1)$$

where \mathbf{u}_i is the velocity of phase $i=b, l$, and \mathbf{K} is the permeability tensor while k_{ri} is the relative permeability of phase i . The pressure is denoted by p and μ_i represents the viscosity of phase i . The saturation (volume fraction) is denoted by a function S with values between 0 and 1 indicating the composition of the mixture of fluids. The coefficients K, k_{ri} are dependent on position in space.

It follows from the mass conservation for each phase that,

$$\text{div } \mathbf{u}_i = q_i, \quad (2)$$

where q_i denotes a source for each phase. After simple manipulations and introduction of new notation for the total mobility,

$$\lambda(S) = \frac{k_{rb}(S)}{\mu_b} + \frac{k_{rl}(S)}{\mu_l}, \quad (3)$$

the following formula, the so-called pressure equation is obtained,

$$-\nabla \cdot (\mathbf{K} \lambda(S) \nabla p) = q, \quad (4)$$

and q denotes the sum of the sources.

In addition to the listed above relations we need to define how the saturation changes due to flow of the fluids mixture. It follows from the summation of the equations (1) that

$$\mathbf{u} = \mathbf{u}_b + \mathbf{u}_l = -\lambda(S) \mathbf{K} \cdot \nabla p. \quad (5)$$

Let us introduce a following notations,

$$F(S) = \frac{k_{rb}(S)/\mu_b}{k_{rb}(S)/\mu_b + k_{rl}(S)/\mu_l}, \quad (6)$$

$$S_t = \frac{\partial S}{\partial t}, \quad (7)$$

and assume that the saturation changes according to the modified advection equation,

$$S_t + \mathbf{u} \cdot \nabla F(S) = 0. \quad (8)$$

The presented here considerations result in the following set of equations,

$$-\nabla \cdot (\mathbf{K} \lambda(S) \nabla p) = q \quad \text{in } \Omega \times [0, T] \quad (9)$$

and

$$S_t + \mathbf{u} \cdot \nabla F(S) = 0 \quad \text{in } \Omega \times [0, T]. \quad (10)$$

This relations were implemented in a computer program based on deal.II library, see (Bangerth, Hartmann and Kanschat, 2007) and a simple case example was calculated and discussed in the next section. In the next step an extension is planned to include poro-elasticity effects in the formulation. Analysis of blood propagation in the surrounding bone fracture tissue enables approximate determination of clot shape and distribution of its density.

Preliminary Results, Discussion and Conclusions

The careful and detailed analysis of the experimental results will provide among the others the indications concerning values of parameters introduced in problem formulation that are required to perform the numerical calculations. Such identification and validation of the theoretical results will be a subject of the next paper. In the present, preliminary investigations we concentrated our attention on the formation and shape of the callus in fractured bones. In Figure 1 an example of RTG pictures for the case with mechanical stimulation and PDGF applied after the 2, 5 and 8 weeks after surgery are presented. The calcification of tissue did not start yet but formation of callus based on clot can be easily observed.



Figure 1. RTG pictures of fractured bone after 2, 5 and 8 weeks. Clot and callus formation are visible.

The formulas discussed in the previous section were implemented in computer program and used in numerical calculations to examine effect of different parameters. Simple 2-D example was considered. Let us consider a rectangle domain, part of porous tissue surrounding fractured bone. Part of the border of this domain is in contact with a bone and a gap from which the blood is leaking to the porous domain. For the needs of this example we assume that no blood source is present inside the

considered domain and that the heterogeneous porous medium is isotropic. The first of these assumptions can be easily justified: there are usually no mechanisms for blood to appear or disappear inside the considered domain. The supply of blood is present only at the fracture region which is located at the part of the border of the considered porous tissue surrounding broken bone. The second one is harder to justify: on a microscopic level, most of bones are anisotropic, because they consist of a network of interconnected pores with ordered orientation. Moreover surrounding bone soft tissue where the blood is leaking and forming clot is partially damaged due to trauma. However, this is impossible to predict in specific cases as we don't know the direction and distribution of cracks which are not accessible. Therefore as the first approximation we make an assumption about isotropy. In future, in the improved formulation this assumption will be possibly dropped. To simplify considerations the numerical simulations were done on the unit cell. In order to make calculations more realistic a set of micro cracks were generated in considered square domain. They determine to big extent permeability of a tissue. The concentration of cracks decreases linearly with distance from the edge of the domain which is in contact with bone. The following initial condition is assumed $S(\mathbf{x}, 0)=0$ in Ω , what is equivalent to an assumption that no blood is present in the domain at the beginning and the pores are saturated with pure liquid. At the border of considered domain we assumed that the blood inflow is only possible at the part of the surface $x_1=0, 0.3 < x_2 < 0.7$ that is at the part of the left edge of the rectangle. Example of calculation results is presented in Figure 2. In this figure time evolution of a distribution of blood (red) and liquid (blue) in considered domain is presented. Such calculations will be used, after a prior identification of values of parameters used in mathematical description, to estimate a domain occupied by a clot and a density distribution of solidified blood. This new solid "structure" serves as a scaffold for the cells involved in tissue regeneration. Therefore such an analysis can be used in modeling of new tissue synthesis, callus formation and bone remodeling phases.

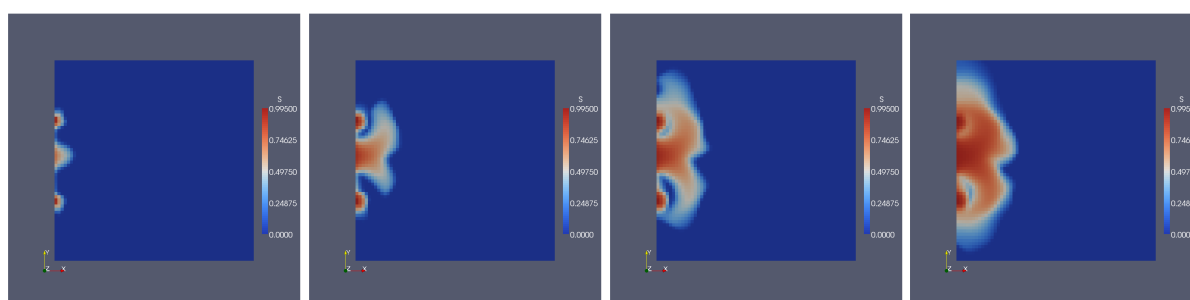


Figure 2. Propagation of blood (red) from the bone fracture to the porous tissue saturated with liquid (blue).

The preliminary results presented in this paper show that even simple model of clot formation applied in numerical simulations may assure interesting and valuable results. The theoretical model still should be verified using clinical observations and the results of mentioned briefly in the previous section experimental investigations. This work will be reported soon in the forthcoming paper.

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