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A meshless Local Boundary Integral Equation (LBIE) method for cell proliferation predictions in bone healing

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Abstract— **Bone healing involves a series of complicated cellular and molecular mechanisms that result in bone formation. Several mechanobiological models have been developed to simulate these cellular mechanisms via diffusive processes. In most cases solution to diffusion equations is accomplished using the Finite Element Method (FEM) which however requires global remeshing in problems with moving or new born surfaces or material phases. This limitation is addressed in meshless methods in which no background cells are needed for the numerical solution of the integrals. In this study a new meshless Local Boundary Integral Equation (LBIE) method is employed for deriving predictions of cell proliferation during bone healing. First a benchmark problem is presented to assess the accuracy of the method. Then the LBIE method is utilized for the solution of cell diffusion problem in a two-dimensional (2D) model of fractured model. Our findings indicate that the proposed here LBIE method can successfully predict cell distributions during fracture healing.**

I. Introduction

Bone fracture healing is a complex regenerative process that gradually restores the functional and mechanical bone properties, such as load-bearing capacity, stiffness and strength. It includes a complex sequence of cellular and molecular events that begin with an inflammatory reaction, lead on to the callus tissue formation, the gradual differentiation of intermediate tissues inside the callus and finally the callus resorption and bone modeling [1]. Despite the intensive work the determination of the underlying cellular mechanisms of bone healing remains an open issue in the literature.

To this end several mechanobiological computational models have been proposed to investigate how mechanical stimulation affects tissue differentiation and biological pathways during the healing course [2, 3, 4]. Most of these models incorporate mechanorgulation algorithms that describe cellular mechanisms by a diffusive process. Lacroix et al., [5] first accounted for combined cell migration, proliferation and differentiation by using diffusion equations to model concentrations of progenitor cells originating from different parts of healing bone i.e., periosteum, bone marrow etc. Bailon-Plaza and van der Meulen [6] were also based on diffusion to model migration and proliferation of mesenchymal cells as well as chondrocyte and osteoblast proliferation and differentiation. More recent mechanobiological models apart from the diffusive cellular processes of tissue differentiation also account for volumetric tissue growth [7, 8] and angiogenesis [9]. Another model presented in [10] the cellular processes are directly connected with mechanical stimulation and act at cell-phenotype rates.

In all the aforementioned studies the diffusion equations are commonly solved using the Finite Element Method. Although FEM is a well-known and robust numerical method, when applied to problems dealing with phase changes, suffers from global remeshing when new born surfaces or material phases appear. To this end, meshless methods have gained significant interest since no background cells are required for the numerical evaluation of the involved integrals [11, 12]. Properly distributed nodal points, without any connectivity requirement, covering the domain of interest as well as the surrounding global boundary are employed instead of any boundary or finite element discretization. All nodal points belong in regular sub-domains centered at the corresponding collocation points. Meshless methods such as the LBIE method [12] and the Meshless Local Petrov-Galerkin MLPG method [13] have been intensively used for the solution of engineering fracture mechanics problems. However no such method has been used to solve diffusion problems describing the cellular proliferation during bone healing.

Very recently a new and simple meshless LBIE method has been proposed for the solution of elastostatic problems [14]. In the present work, the LBIE methodology of [14] is properly modified to model cell proliferation during bone healing via a diffusive process. First we assess the effectiveness of the method via a representative simple benchmark problem by comparing the numerical results derived from LBIE with those derived theoretically. Then LBIE method is employed to derive cell predictions in bone healing by considering a 2D model of fractured bone. The model included initial cell concentrations at the periosteum, the marrow and between bone and callus (at the fracture end). Numerical predictions of cellular distribution in the callus region are performed for 25 days post-fracture.

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II. Materials And Methods

A. Theoretical development of cell diffusion

As it is pointed out in [5], mesenchymal cells (MSCs) coming mainly from the bone marrow and periosteum differentiate into fibroblasts, osteoblasts and chondrocytes and generate fibrous tissue, cartilage and new bone material. The dynamics of the MSCs is usually described by complicated and nonlinear systems of partial differential equations that correlate all the types of cells that take part in bone healing process [14]. A simplified, but very effective model is to consider the concentration of all types of cells as as $c(\mathbf{x},t)$ satisfying the diffusion equation

$$\partial_t c(\mathbf{x},t) = D\nabla^2 c(\mathbf{x},t) + f_{pr}c(\mathbf{x},t) - F_{dif} - F_{ap}. \quad (1)$$

∂_t indicates differentiation with respect to time, $\mathbf{x}$ is the position vector of a point in a two dimensional space, ∇ represents the gradient operator, D is the diffusivity of the cells, and f_{pr}, F_{dif}, F_{ap} parameters that regulate rates of proliferation, apoptosis and differentiation, respectively. Considering the finite difference scheme

$$\partial_t c^{k+1} = (c^{k+1} - c^k)/\Delta t + O(\Delta t),$$

(1) is written for the time step $k+1$ as

$$\nabla^2 c^{k+1}(\mathbf{x},t) = (\frac{1}{D\Delta t} - \frac{f_{pr}}{D})c^{k+1}(\mathbf{x},t) + \frac{F_{dif}+F_{ap}}{D} - \frac{1}{D\Delta t}c^k(\mathbf{x},t). \quad (2)$$

The boundary conditions of the problem are assumed to be

$$\left.\begin{array}{c} c(\mathbf{x},t) = \overline{c}(\mathbf{x},t),\ \mathbf{x}\in S_1 \\ \partial_n c(\mathbf{x},t) = \overline{q}(\mathbf{x},t),\ \mathbf{x}\in S_2 \\ S_1 \bigcup S_2 \equiv S, \end{array}\right\} \quad (3)$$

with S, n being the global boundary surrounding the fractured area and its normal vector, respectively and $\overline{c}(\mathbf{x},t), \overline{q}(\mathbf{x},t)$ prescribed functions.

B. Meshless LBIE formulation

The aforementioned boundary value problem is solved here through a meshles LBIE method, described in brief in what follows. More details one can find in [11, 12,14].

Consider the domain of fractured bone V surrounded by a closed boundary S and a set of arbitrarily distributed and without any connectivity requirement points that covers the domain V and called nodal points. The global boundary S is represented by a group of points imposed by a boundary element mesh. The nodes of the boundary element mesh are considered also as nodal points of the problem. Each nodal point $\mathbf{x}^{(k)}$ is located at the center of a circular area Ω_s with boundary $\partial\Omega_s$ called support domain of $x(k)$. Then the cell concentration at that point admits an integral representation of the form:

$$\begin{aligned} &\alpha c^{k+1}(\mathbf{x}^{(k)},t) + \int_{\Gamma_s\cup\partial\Omega_s} \partial_n\Phi^*(\mathbf{x}^{(k)},\mathbf{y})\cdot c^{k+1}(\mathbf{y},t)dS_{\mathbf{y}} = \\ &\int_{\Gamma_s}\Phi^{**}(\mathbf{x}^{(k)},\mathbf{y})\cdot\partial_n c^{k+1}(\mathbf{y},t)dS_{\mathbf{y}} - \int_{\Omega_s}\Phi^{**}(\mathbf{x}^{(k)},\mathbf{y})\cdot\left(\frac{1}{D\Delta t}-\frac{f_{pr}}{D}\right)c^{k+1}(\mathbf{y},t)dV_{\mathbf{y}} - \\ &\int_{\Omega_s}\Phi^{**}(\mathbf{x}^{(k)},\mathbf{y})\cdot\left(\frac{F_{dif}+F_{ap}}{D}-\frac{c^k}{D\Delta t}\right)dV_{\mathbf{y}} \end{aligned} \quad (4)$$

where the coefficient α equals to 1 when $\mathbf{x}^{(k)}$ is internal point and ½ when $\mathbf{x}^{(k)}$ lies on the global boundary S. Γ_s represents the portion of the global boundary when it is intersected by the support domain Ω_s, while the kernels Φ^*, Φ^{**} have the form:

$$\begin{aligned} \Phi^*(r) &= -\frac{1}{2\pi}\ln r, \\ \Phi^*(r) &= -\frac{1}{2\pi}\ln r_0, \end{aligned} \quad (5)$$

with r representing the distance $r = |\mathbf{x}-\mathbf{y}|$ and r_0 the radius of the support domain.

Interpolating the concentration functions through Radial Basis Functions (RBF) and collocating the LBIE (4) at all nodal points, as it is explained in detail in [11] and [14] the following system of algebraic equations are obtained:

$$\mathbf{H}\cdot\mathbf{c}^{(k+1)} + \mathbf{G}\cdot\mathbf{q}^{(k+1)} = \mathbf{b}^{(k)}, \quad (6)$$

where the vector $\mathbf{c}^{(k+1)}$ comprises all the nodal values of the concentration functions, $\mathbf{q}^{(k+1)}$ is a vector consisting of all nodal fluxes, the flux $\partial_n c^{(k+1)}$ is defined only on the global boundary and the vector $\mathbf{b}^{(k)}$ contains all the known quantities from the previous step.

Applying the boundary conditions (3) the system (6) obtains eventually the form

$$\mathbf{A}\cdot\mathbf{z} = \mathbf{b}, \quad (7)$$

with the vector $\mathbf{z}$ comprising all the unknown nodal concentrations and boundary concentrations fluxes and $\mathbf{b}$ containing all the known nodal quantities. This system can be solved easily and efficiently through a typical **LU** decomposition solver since, due to the local nature of the method, the matrix **A** is sparse and banded.

In order to test the accuracy of the present formulation, a benchmark problem is first solved and the results are compared with the analytical solution following the equation:

$$c(y,t) = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{n+1}\exp\{-\frac{(2n+1)^2\pi^2\kappa t}{4L^2}\}\cos\frac{2n+1}{2L}\pi y, \quad (8)$$

where L is the diffusion length and κ is the diffusivity, both being assigned with unit values.

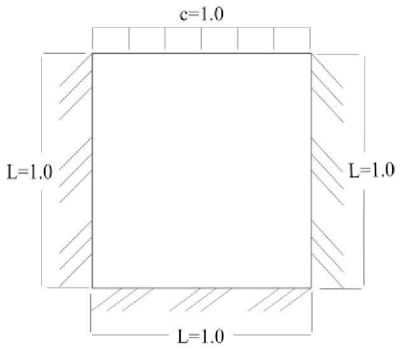

Figure 1. Geometry of the cross section of a unit cube with initially zero cell concentration subjected to a sudden concentration increase on one of its faces.

Consider the cross section of a unit cube with initially zero cell concentration subjected to a sudden concentration increase on one of its faces while the other three sides remain insulated (Fig. 1).

The diffusitivity coefficient is assumed to be such that $\kappa = 1.81$ internal and boundary nodal points have been used for the solution of the problem via the aforementioned meshless LBIE method. The obtained results are compared to analytical ones (8) and both are depicted in Fig. 2. It can be seen that the agreement is excellent.

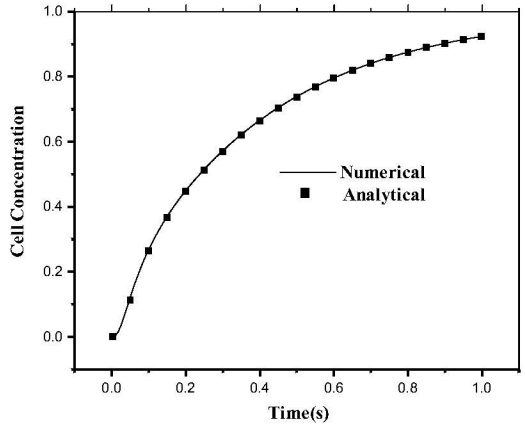

Figure 2. Cell concentration as a fucntion of time calculated from the meshless LBIE method. The theoretical solution taken from (8) is also presented with the dotted line.

C. Geometrical model of healing bone

We assumed a model of callus based on 2D mechanical models of healing bone in a previous study [10] as depicted in Fig.3. The cortical bone had inner and outer diameters of 14 mm and 20 mm [10]. Cell parameters were considered equal to those of mesenchymal stem cells (MSC). The diffusivity was equal to $0.65 mm^2$/day whereas the proliferation, differentiation and apoptosis rates were 0.6 mm^2/day, 0.3 mm^2/day and 0.05 mm^2/day respectively [10].

The initial conditions in the cell model included concentrations of MSC at the periosteum, the marrow interface and the interface between bone and callus at the fracture site as shown in Fig 2. In addition the flux across the remainder external boundaries was constrained by assuming $\frac{dc}{dn} = 0$ (Fig. 3). Numerical calculations were performed for 300 iterations which correspond to 25 days post-fracture. The interior of the callus is fulfilled with 589 distributed nodes.

III. Results

Figure 4 presents the numerically predicted MSC distribution in the callus area at days 8, 16 and 23 after fracture. Note that red colors correspond to maximum cell concentrations whereas deep blue ones to minimum. It can be seen that at day 8 (Fig. 4 (a)) mesenchymal cells proliferation has started in the fracture gap. Increased MSC concentrations are also found along the periosteum at some distance from the gap. Subsequently at days 16 and 24 i.e., Figs. 4(b), (c) cells proliferate in the periosteum covering first the remote fracture site. Concurrently cell concentrations are significantly increased in the fracture gap at day 24, which

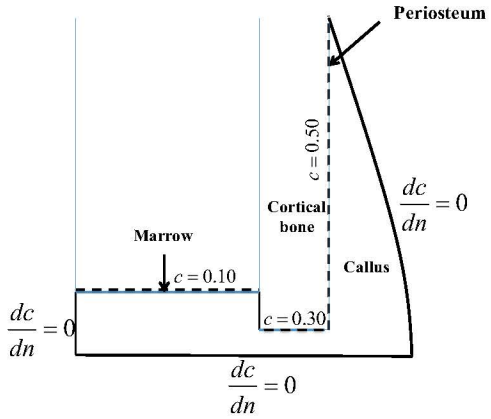

Figure 3. The geometry of the 2D model of healing bone. Initial MSC concentrations were included at periosteum, marrow and at the interface between bone and callus.

suggests that progressive intramembraneous ossification occurs possibly followed by endochondral replacement.

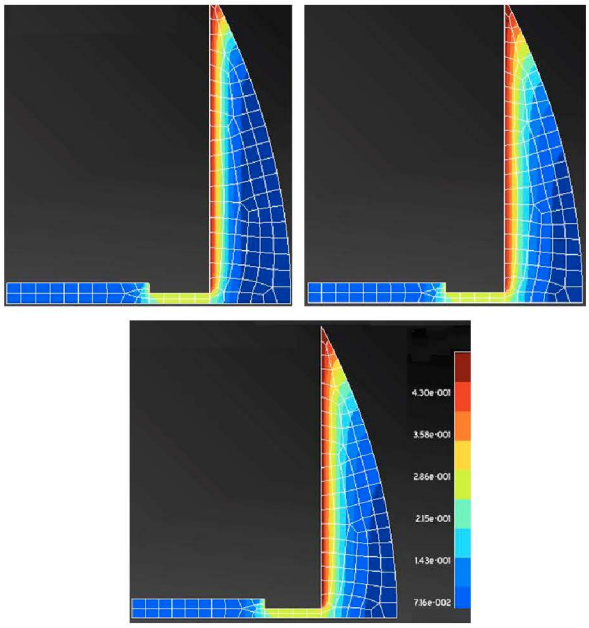

Figure 4. Predicted distributions of MSC in the callus during normal fracture healing at (a) day 8, (b) day 16 and (c) day 23.

IV. Discussion

In this work a new meshless LBIE method was applied for the solution of the diffusion problem that models cell proliferation during bone healing. Simulations of MSC distribution were performed in a computational model of healing bone for 25 days post fracture.

Since biological cell processes play a key role in bone healing, they have been incorporated in several mechanobiological models of tissue differentiation [5-10]. However, in most cases the description of cell activities is achieved by complicated and nonlinear systems of partial differential equations that correlate all the types of cells that take part in bone healing process. In this study we adopted a

simplified linear but rather effective model in which the concentration of all cell types is modeled as one parameter following a diffusion equation reported in [10]. Cell proliferation, differentiation and apoptosis were also taken into account. Nevertheless the diffusion model presented herein is suggested as a first approach to describe cell proliferation and also for investigating whether an LBIE method could provide reasonable predictions in such biological problems. The incorporation of non-linearity issues in the model is still in progress and the results will be presented in future publications.

Although FEM is the most common method used in mechanobiological studies for deriving numerical solution to cell diffusion problems, it has several limitations associated with global remeshing as phases change. This can be addressed by the use of meshless methods in which properly distributed nodal points, without any connectivity requirement, cover the domain of interest as well as the surrounding global boundary. Neverhteless no such method has been previously applied to calculate cell diffusion during bone healing. This has been done n the present study where a new and very accurate meshless LBIE method is proposed for a solution of such diffusion problems.

The accuracy of a Local Boundary Integral Equation (LBIE) method, which utilizes compactly supported Radial Basis Functions (RBFs) or Local RBFs for the interpolation of the considered fields can be affected negatively when: (i) The positive definitiveness of RBFs is not guaranteed, (ii) the interpolation utilizes derivatives of RBFs, and (iii) the size of support domains is not the optimum one.

In the present LBIE formulation, the positive definitiveness of RBFs is accomplished with the use of additional polynomial terms in the definition of RBFs, no derivatives of RBFs are employed and the size of the support domains is taken in an optimum way since the support domain of each point intersects the support domains of all the other points that belong to the support domain of the point.

Thereafter we performed simulations in a 2D model of callus so as to investigate MSC proliferation and calculate cell concentration. MSCs were assumed to come from the bone-callus interface, the bone marrow and the periosteum.

The results showed that various characteristic events of bone healing could be captured. MSC activity at the fracture gap started from the 8^{th} day and was progressively increasing. Therefore MSCs differentiate into fibroblasts leading to the formation of fibrous tissue close to the fracture ends. Concurrently at 8^{th} day cells started proliferate along the periosteum at a distance from the fracture gap, which may be indicative of an initial intramembraneous bone formation. Cell proliferation was also found to successively increase at the regions closer to the fracture gap possibly towards the development of cartilage.

Our findings make clear that meshless LBIE method provides reasonable predictions of cell distributions during bone healing. However in order to draw accurate conclusions about the cellular activities, the diffusion model needs to be enhanced so as to incorporate cell-phenotype specific activities.

V. Conclusions

We presented a new meshless LBIE method for deriving predictions about MSC concentration during bone healing. Numerical simulations of cell diffusion were performed in a 2D computational model. An increased cell activity was observed at the areas close to the fracture site suggesting the progressive formation of fibrous tissue. Furthermore intensive cell action was also observed along the periosteum suggesting an initial intramembraneous bone formation starting from a distance from the fracture gap. Overall meshless LBIE method can capture significant events that occur during bone healing and could be thus considered as a significant tool for solving diffusion problems. However isolated cell parameters need to be analyzed and compared with experimental findings so as to accurately interpretate our findings.

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