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A CFD Level-Set Method for soft tissue growth: theory and fundamental equations

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Abstract

A level-set method, specifically conceived for the case of soft organic tissue growth from feeding solutions, is introduced and described in detail. The model can handle the morphological evolution of the organic specimen under the influence of external convection (fluid-dynamics of the bioreactor). The analogies and differences between this technique and a previous volume of fraction method are discussed pointing out advantages and limitations of both formulations.

Categories:

65C20: Models, numerical methods
76Z99: Biological Fluid Mechanics
76D05: Navier-Stokes equations
92C45: Kinetics in Biochemical Problems
92C05: Biophysics
74N20: Dynamics of phase boundaries

Introduction

Tissue engineering often studies the process of building tissue like constructs ex vivo that will have eventual use for implantation in vivo. The ultimate target of a tissue engineered product is to replace or augment existing tissue function. Common techniques of building tissue engineered products include seeding cells on either degradable or inert material scaffolds, layering cells in composite constructs, or building up cell populations in vitro through traditional culture flask techniques. Recently, a new rotary culture vessel technology has been developed (see e.g. the excellent overview of Hammond and Hammond, 2001) that minimizes the fluid shear forces that are often problematic in stirred bioreactors. This new technology allows stable cell constructs to form on microcarrier beads in the media and can offer insight into the early phases of tissue morphogenesis in vivo.

Further progress in creating proper environments for the growth of the tissues requires an understanding of how chemical, mechanical and other environment factors influence growth. Within this context, it is worthwhile to stress how the available "mechanical" theories (see e.g. the excellent models of Taber, 1998) have focused on "what happens inside the tissue"; on the contrary numerical methods for simulating the biomechanical laws that govern soft tissue growth in terms of "surface incorporation/conversion conditions" (interface kinetics of the growth) remain still poorly developed. In practice from a numerical point of view, the growing biological specimen gives rise to a moving boundary problem. Moving boundary problems remain a challenging task for numerical simulation, prompting much research and leading to many different solutions.

Volume tracking methods (e.g. volume of fluid (VOF), SLIC simple line interface calculation, and PLIC piecewise linear interface calculation), have became popular in the last years for the simulation of many technological problems dealing with moving interfaces (for a very comprehensive discussion dealing with the genesis and the evolution of these phase-field methods see Hirt and Nichols, 1981; Gueyffier, Li, Nadim, Scardovelli, Zaleski, 1999; Rider and Kothe, 1998 and references therein). In particular, they have been used for the simulation of typical industrial problems associated to

gas/liquid or liquid/liquid systems where the surface tension effects play a ‘critical role’ in determining the shape of the fluid/fluid interface and/or its motion.

On the other hand, ‘enthalpy methods’ and similar techniques taking into account the release or absorption of latent heat have been successfully applied to the case of thermal phase change problems characterized by the presence of moving solid/melt interfaces due to the heating or the cooling of the system under investigation (see e.g. Bennon and Incropera, 1987).

Recently these approaches have been extended to other types of "growth" that are very different with respect to the case of solidification of melts i.e. the case of macromolecular organic growth from "supersaturated solutions" (due to the addition/incorporation of solute molecules (building blocks or growth units) to the crystal lattice) and the growth of biological tissues in bioreactors (Lappa, 2003a). For instance such methods were used by Lappa (2003b; 2003c) to discern the relative importance of surface kinetics and mass transport as "limiting steps" for the growth rate of lysozyme crystals; Lappa (2003d) investigated the evolution of the solid/liquid interface of a single sample of soft tissue surrounded by the nutrient solution focusing on the surface metabolism and its sensitivity to many "local" environmental factors.

It is worthwhile to point out how, in addition to the above-cited worthy VOF-based contributions, Level-set methods are recently enjoying a widespread use and are becoming the most popular techniques for the simulation of moving boundary problems (see e.g. the landmark works of Osher and Sethian, 1998; Sussman, Smereka and Osher, 1994; Osher and Fedkiw, 2002; Sethian, 1999) . The great success of level set methods can be attributed to their versatility and simplicity as well as to significant advantages offered in taking into account some aspects (Osher and Fedkiw, 2002, Sethian. 1999). For these reasons, the methodology and the formalism underlying modern level set methods has been extended to the aforementioned techniques dealing with the solidification of melts (see e.g. the excellent analysis of Chen, Merriman, Osher and Smereka, 1997).

Along these lines, the objective of the present analysis is to re-formulate the (VOF) kinetic model (proposed by Lappa, 2003a, 2003d in the case of tissue growth) in the frame of a Level-Set technique.

The surface kinetics of tissue growth

As pointed out in Lappa, (2003d) where an exhaustive model of the soft tissue surface kinetics was introduced and validated through comparison with experimental results, the paradigm equation governing the aforementioned kinetics must take into account the main aspects of the growth behaviour for biological tissues, i.e. the availability of nutrients, the slow surface absorption mechanisms and the effect of surface shear stress:

$$\left.\frac{\partial C}{\partial n}\right|_i = \bar{f}(C_i, \tau, \lambda, D) \qquad (1)$$

where $\bar{f}$ is a function depending on the type of tissue, C_i is the concentration of the nutrient at the construct/liquid interface; D is the diffusion coefficient of the nutrient in the feeding solution, λ is a 'kinetic coefficient' having the dimensions of a velocity (e.g. [cm/s]) and τ is the fluid-dynamic shear stress at the tissue/liquid interface (*n* denotes direction perpendicular to the advancing tissue surface). From mass balance the non-dimensional velocity (q_n) of the advancing tissue interface reads :

$$q_n = \frac{\rho_T}{\rho_S} f(C_i, \tau, \lambda, D) \qquad (2)$$

where ρ_T is the mass density of the tissue, ρ_S is the total density of the feeding liquid and f is the non-dimensional form of $\bar{f}$. The non-dimensional form of the equations results from scaling the lengths by a reference distance (L), the time by L^2/D, velocity $\underline{V}$ and pressure p by D/L and $\rho_S D^2/L^2$ respectively and the solute concentration by its initial value $C_{(o)}$. By analogy with the case of protein crystal growth, q_n can be also seen as the "growth rate" of the biological tissue.

The OTGLSET - Organic Tissue Growth Level Set Method

General properties

This method, first introduced by Osher and Sethian (1998), is conceptually similar to a phase-field model in that the solid–liquid interface Γ is represented as the zero contour of a level set function, $\varphi(\underline{r},t)$, which has its own equation of motion. The movement of the interface is taken care of implicitly

through an advection equation for $\varphi(\underline{r},t)$. Unlike the phase-field model, however there is no arbitrary interface width introduced in the level set method; the sharp-interface equations can be solved directly and, as a result, no interface reconstruction techniques (e.g. PLIC) are required.

The goal is to compute and analyze the subsequent motion of Γ under a velocity field q (Osher, Fedkiw, 2002). This velocity can depend on position of the interface of the organic construct (Ω) and the external physics (for the case under investigation it depends on the surface incorporation kinetics i.e. on the mechanisms of incorporation of solute molecules into the growing solid surface i.e. eq. (1)). The tissue boundary is captured for later time as the zero level set of the function $\varphi(\underline{r},t)$, i.e., $\Gamma(t) = \underline{r}\big|_{\varphi(\underline{r},t)=0}$. In practice the level set function is defined as the signed normal distance from the solid–liquid interface such that φ is positive in the liquid phase, negative in the tissue, and zero at the solid/feeding-solution interface:

$$\begin{aligned} &\varphi(\underline{r},t)>0 \quad \text{for } \underline{r}\notin\Omega \\ &\varphi(\underline{r},t)=0 \quad \text{for } \underline{r}\in\partial\Omega=\Gamma(t) \\ &\varphi(\underline{r},t)<0 \quad \text{for } \underline{r}\in\Omega \end{aligned} \tag{3}$$

Thus, the specimen front is to be captured for all later time, by merely locating the set $\Gamma(t)$ for which φ vanishes. Its motion is analyzed by convecting the φ values (levels) with the velocity field $\underline{q}$:

$$\frac{\partial\varphi}{\partial t} + \underline{q}\cdot\underline{\nabla}\varphi = 0 \tag{4}$$

Here $\underline{q}$ is the desired velocity on the interface, and is arbitrary elsewhere.

Actually, only the normal component of $\underline{q}$ is needed: $q_n = \underline{q}\cdot\frac{\underline{\nabla}\varphi}{|\underline{\nabla}\varphi|}$ so eq. (4) becomes

$$\frac{\partial\varphi}{\partial t} + F|\underline{\nabla}\varphi| = 0 \tag{5}$$

Integrating Eq. (5) for one time step results in moving the contours of φ along the directions normal to the interface according to the velocity field F, which varies in space. F is constructed to be an extension of the interface velocity, q_n , such that $F=q_n$ for points on the interface and the lines of

constant F are normal to the interface (see eq. (7)). Thus, advecting φ according to Eq. (5) moves the front with the correct velocity.

After solving Eq. (5) for one time step, the level set function will no longer be equal to the distance away from the interface. It is necessary to *reinitialize* φ to be a signed distance function. This step is accomplished by solving

$$\frac{\partial \psi}{\partial \tau} + sqn(\varphi)\left[\left|\underline{\nabla}\psi\right| - 1\right] = 0 \qquad (6)$$

$$\psi(\underline{r},0) = \varphi(\underline{r},t)$$

Following Osher and Fedkiw (2002), in order to define the distance φ in a band of width ε around $\Gamma(t)$, eq. (6) is solved only for $\tau = O(\varepsilon)$.

The basic level set method concerns a function φ(r,t) which is defined throughout space. Clearly this is useless if one only cares about information near the zero level set. The local level set method defines φ only near the zero level set. In practice, eq. (5) is solved in a neighbourhood of $\Gamma(t)$ of width $m\Delta r$, where m is typically 5 or 6. Points outside of this neighbourhood need not be updated by this motion. The function φ *is* reinitialized to be signed distance to $\Gamma(t)$, only near the boundary, smoothly extending the velocity field q_n off of the front $\Gamma(t)$ (see eq. (7)) and solving equation (5) only locally near the interface $\Gamma(t)$, thus lowering the complexity of this calculation by an order of magnitude (Osher and Fedkiw, 2002). This makes the cost of level set methods competitive with other techniques.

With regard to the smooth extension of the quantity q_n on $\Gamma(t)$ to a neighbourhood of $\Gamma(t)$, this step is accomplished by solving:

$$\frac{\partial F}{\partial t} + sqn(\varphi)\left[\frac{\underline{\nabla}\varphi}{\left|\underline{\nabla}\varphi\right|} \cdot \underline{\nabla} F\right] = 0 \qquad (7)$$

$$F(\underline{r},0) = q_n(\underline{r},t)$$

Again, this equation is solved only for $\tau = O(\varepsilon)$ in order to extend q_n to be constant in the direction normal to the interface in a region of width ε.

For the case under investigation q_n is given by eq.(2) with C at the specimen surface satisfying eq. (1). It is worthwhile to stress how the function q_n is not given "a priori" but has to be computed at any instant as part of the problem (it dynamically changes during the growth process according to the residual nutrient concentration available in liquid phase, according to the steepness of the concentration gradient at the solid/liquid interface that in turn is changed by the motion of the fluid surrounding the macromolecular seed and according to the intensity of the fluid-dynamic shear stress that is not constant in time). For further details on the computation of q_n see the next section.

Governing field equations:

In presence of convection (for instance in the case of rotating bioreactors, convection is driven by the dynamic endless sedimentation of the scaffolds in the feeding liquid, see e.g. Hammond and Hammond, 2001 and Lappa, 2003d), the flow is governed by the continuity, Navier-Stokes and species equations, that in non-dimensional conservative form read :

$$\underline{\nabla} \cdot \underline{V} = 0 \tag{8}$$

$$\frac{\partial \underline{V}}{\partial t} = -\underline{\nabla} p - \underline{\nabla} \cdot [\underline{V}\underline{V}] + S_c \, \nabla^2 \underline{V} \tag{9}$$

$$\frac{\partial C}{\partial t} = \left[- \underline{\nabla} \cdot (\underline{V} C) + \nabla^2 C \right] \tag{10}$$

where V is the fluid velocity, p the pressure and $S_c = \upsilon / D$ is the Schmidt number, (ν is the kinematic viscosity of the culture liquid).

Equations (8)-(10) are not solved for the domain occupied by solid phase since there convective velocities are zero

On the surface of the organic construct (φ=0), the concentration must satisfy the kinetic condition (eq.(1)); with regard to this aspect note that since $\frac{\partial C}{\partial n} = \underline{\nabla} C \cdot \underline{\hat{n}}$, where $\underline{\hat{n}} = \frac{\underline{\nabla} \varphi}{|\underline{\nabla} \varphi|} = (\alpha, \beta)$ is the unit vector perpendicular to the tissue surface

$$\alpha = \frac{\partial \varphi}{\partial x} / \sqrt{\left(\frac{\partial \varphi}{\partial x}\right)^2 + \left(\frac{\partial \varphi}{\partial y}\right)^2} \qquad \beta = \frac{\partial \varphi}{\partial y} / \sqrt{\left(\frac{\partial \varphi}{\partial x}\right)^2 + \left(\frac{\partial \varphi}{\partial y}\right)^2} \tag{11}$$

equation (1) can be re-written as (hereafter the subscript 'i' is omitted):

$$\alpha \frac{\partial C}{\partial x} + \beta \frac{\partial C}{\partial y} = f(C, \tau, \lambda, D) \tag{12}$$

where $f(C, \tau, \lambda, D)$ represents the mass exchange flux between solid and liquid phase (i.e. tissue and nutrient medium) driven by the surface kinetics of the specific tissue under consideration. Finally the interface velocity is computed as $q_n = (\rho_T / \rho_S) f$ (eq.(2)).

Solution procedure

The solution procedure is summarized in the flow-scheme below; it proceeds in 5 major stages:

[i] advancing the interface (eq. (5)), [ii] reinitializing the level set function to be a signed distance function (eq.(6)), [iii] solving for the new concentration and velocity fields in the liquid (eqs. 8-10), [iv] adjourning of the local values of C at the tissue/feeding-solution interface (eq. (12), this stage accounts for solute depletion), [v] computing the surface growth rate distribution (eq. (2), the growth velocity is not directly imposed but it results from internal conditions related to solute transport).

The Navier Stokes equations can be solved using the SMAC method (it is not described here since it is well-known, for further details see e.g. Lappa, 2003d or Lappa, 2003b for the case of macromolecular crystal growth). The domain can be discretized with a uniform mesh with the flow field variables defined over a staggered grid.

Discussion and comparison with the OTGVOF method

It is worthwhile to compare the present OTGLSET with the corresponding "phase field" (VOF) method introduced by Lappa (2003d) in order to show analogies and differences, advantages and limitations.

The OTGVOF method is a "volume of fraction" method. It accounts for the solid mass stored in the generic computational cell by assigning an appropriate value of a phase field variable (ϕ) to each mesh point (ϕ=1 biological tissue, ϕ=0 feeding solution and $0<\phi<1$ for an interfacial cell). The key element for the OTGVOF method is its technique for adjourning ϕ. Upon changing phase, the ϕ-value of the cell is adjusted to account for mass increase, this adjustment being reflected in the nutrients concentration distribution as a sink. The modelling of these phenomena leads to the introduction of two differential equations, strictly related, from a mathematical point of view, to the 'kinetic conditions' used to model mass transfer at the tissue surface. The first equation is eq. (12), the second one is a phase field equation where the mass exchange flux $f(C,\tau,\lambda,D)$ between the specimen and the solution is used to update the value of ϕ in the computational cell located "astride" the tissue front (see Lappa, 2003d). According to these equations, if the nutrients concentration is locally depleted, correspondingly, the solid mass stored in the computational cell grows and the phase variable is increased.

The present technique (OTGLSET) leaves aside mass exchange phenomena i.e. it does not take into account the solid mass stored in each computational cell and the evolution equations for this quantity. Rather the surface kinetics are directly used to compute the normal velocity at the organic-construct/liquid interface (i.e. the "growth rate"). Then this velocity is used to advect φ and calculations of this function are performed only in a narrow region around the interface as previously pointed out.

If the nutrients concentration is locally depleted, correspondingly, the concentration gradient in eq. (1) and the associated growth rate in Eq. (2) are positive and the interface Γ is transported outward.

Although the volume of fraction model has led to very interesting results for the case of protein crystallization and tissue growth (see Lappa 2003b,2003c,2003d), there are still some limitations in this approach. The proper use of these models requires in fact that an asymptotic analysis be performed in order to obtain a mapping between the parameters of the phase-field equations and the sharp-interface equations i.e. a very accurate "interface reconstruction technique is required" (for

further details on this aspect see Lappa 2003b, 2003c). Moreover computationally, the grid spacing must be small enough to resolve the interfacial region. The multiphase region (region where phase change occurs i.e. region where the "growth units" are incorporated in the pre-existent specimen and converted into its main components) is in fact defined by the condition $0<\phi<1$ and is therefore associated to a somehow arbitrary thickness (the "width" depends on the resolution of the computational mesh).

A reconstruction is a geometrical approximation of the true solid/liquid interface (to be applied in the computational cells where $0<\phi<1$), and various techniques are available in literature for this purpose. For the OTGVOF method described in Lappa (2003a and 2003d), the interface was approximated by a straight line of appropriate inclination in each cell (PLIC piecewise linear interface approximation): for this case the slope of the line is given by the interface normal (gradient of the volume fraction ϕ), and the intercept follows from invoking volume conservation. The reconstructed interface then was used to compute the fluxes necessary to integrate the volume evolution equation.

These techniques are very laborious and not easy to implement (for a technical description of the PLIC approach see the excellent analysis of Gueyffier et al., 1999). On the contrary, the level set computational approach has the capability to track the motion of the interface without resorting to mathematical manipulations and complex reconstruction techniques. Moreover there is not any arbitrary thickness associated with the region where liquid turns to solid since the interface is captured as the zero level set of the function (it is sharp). Shape changes, corner and cusp development, and accurate determination of geometric properties are naturally obtained in this setting. Moreover topological merging and breaking are well defined and easily performed.

The results provided by the present level set method have been compared with simulations of tissue growth performed using the phase-field model. Figs.1 show level-set-based simulations for the same conditions dealing with the growth of cartilage construct ($f \propto \frac{L\lambda}{D}(\tau)^{1/2}(C)$) that was simulated by Lappa (2003a and 2003d). The results exhibit a satisfactory agreement.

Conclusions

In conclusion the level set method should be considered as an interesting alternative to the use of volume of fraction models for the simulation of problems dealing with the growth of biological tissues and the related slow surface kinetics. The level set method offers significant advantages in terms of versatility, robustness and simplicity and exhibits wide capabilities to provide all the parameters of interest for organic tissue growers.

Acknowledgements

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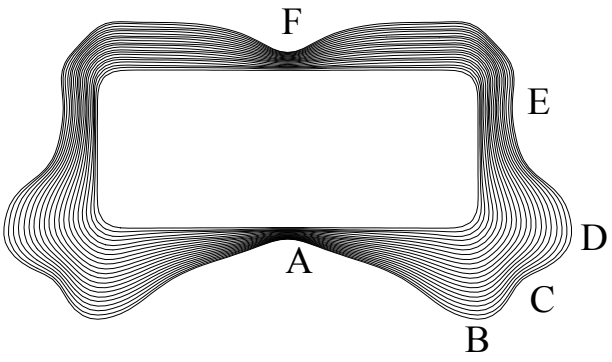
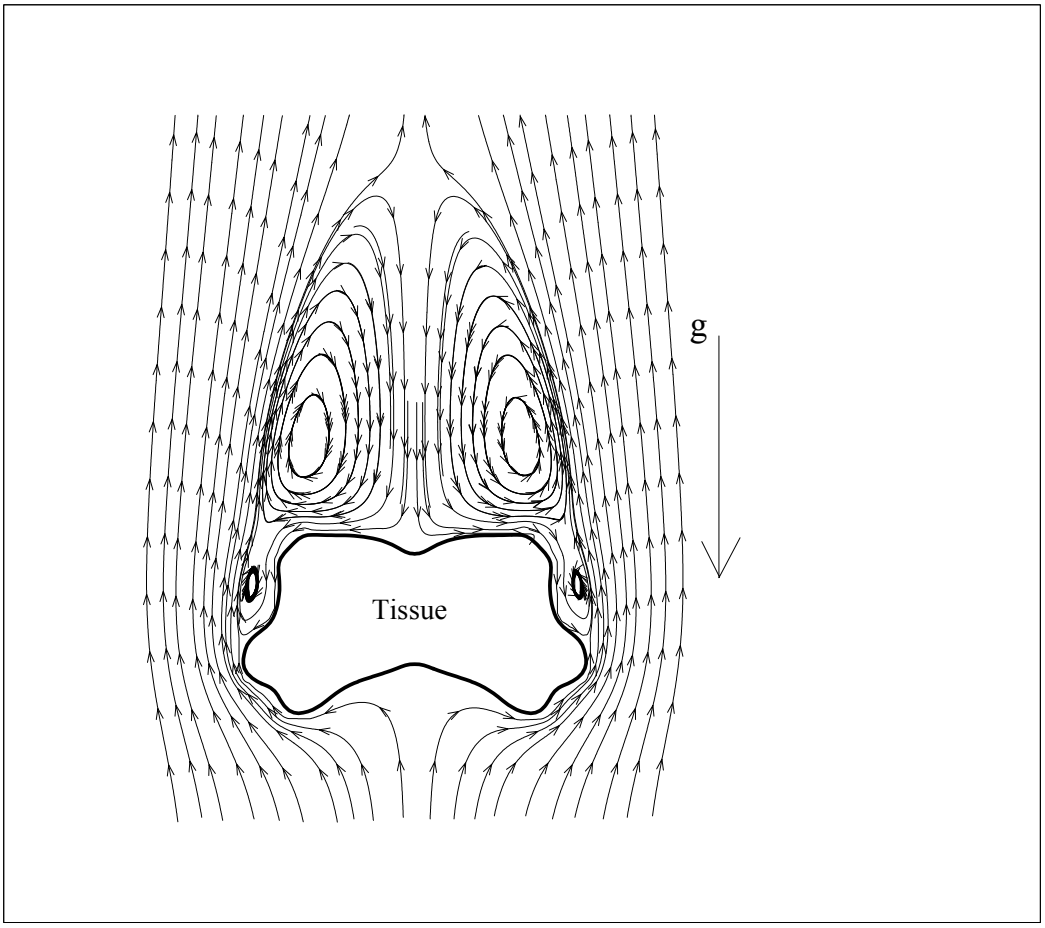


a)



b)

Fig. 1: (a) Tissue growth habit simulation and progression of cartilaginous matrix deposition: snapshots of the tissue shape versus time ($\Delta t = 5.2 \cdot 10^4$ [s]); (b) Cartilage tissue and surrounding velocity field after 40 days (to be compared with the corresponding figure in Lappa, 2003d). The data used for the simulations correspond to the environment provided by a a rotating-wall perfused vessel and in particular to the experimental conditions described in the article Obradovic et al., (2000) and previously simulated by means of a VOF method by Lappa (2003a and 2003d).