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Biological Tissue Growth in a Double-Scaffold Configuration

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Abstract: Numerical simulations and computer-graphics animation can be used as useful tools to discern the physicochemical environmental factors affecting the surface kinetics of growing biological tissues as well as their relative importance in determining growth. A mathematical formalism for such kinetics is proposed through parametric investigation and validated through focused comparison with experimental results. The study relies on the application of a CFD moving boundary (Volume of Fluid) method specially conceived for the simulation of these problems. In the second part of the analysis the case of two samples hydrodynamically interacting in a rotating bioreactor is considered. The interplay between two specimens in a tandem arrangement is investigated in terms of the shear stress environment that occurs around the trailing scaffold due to the fluid-dynamic wake released by the leading one and in terms of the consequent construct morphological evolution.

keyword: Tissue Engineering, VOF methods, Growth Kinetics.

1 Introduction

Tissue engineering has been defined as the application of principles and methods of engineering and life sciences for the development of biological substitutes, to restore, maintain or improve tissue function (i.e. the introduction of the tools and thinking of mechanical engineering into tissue physiology). The in vitro culture of three-dimensional (3D) tissues under conditions that support efficient nutrition of cells, possibly combined with the application of mechanical stimuli to direct cellular activity, is an important step towards the development of functional grafts for the treatment of lost or damaged body parts (i.e. functional tissue engineering). Among other things, engineering 3D tissues in vitro could also yield non-implantable structures to be used as external organ support devices; furthermore, engineered tissues could provide reliable model systems, facilitating a fundamental understanding of structure–function relationships in normal and pathological conditions (see, e.g., Martin *et al.*, 2004).

The generation of 3D tissues ex vivo, however, not only poses new technical challenges owing to the physicochemical requirements of large cell-masses but also requires the development of new biological models (rather than those already established). Major obstacles to the generation of functional tissues and their widespread clinical use, in fact, are still related to a limited understanding of the regulatory role of specific mechanical and physicochemical culture parameters on tissue development.

Understanding the biochemistry of growth is most desirable. Biochemistry can explain *why* a tissue grows. That is not enough, however. It is also necessary to know *how* a tissue grows. The latter means macroscopic description in terms of the macroscopically measurable parameters.

The macroscopic parameters of tissue growth seem to be self-evident: *mass* and *form* (*configuration*). Hence, last but not least is the understanding of the process of spatial pattern formation. As outlined before, it is very likely that we can affect these processes by applying mechanical stimuli. The working hypothesis that has driven many works over recent years, in fact, is that although tissue morphogenesis tends to be genetically-determined and chemically mediated, the actual process of tissue construction may be regulated *mechanically.*

Many models have appeared where this topic was addressed by engineers in the framework of theories dealing with the mechanics of elastic bodies and fibers. According to many investigators (see, e.g., Klisch *et al.*, 2001, Taber 1998a,b), for instance, growth and remodeling in tissues may be modulated by mechanical factors such as internal stress.

Since conventional static tissue culture methods form flat sheets of growing cells (settled on the bottom of the container) that differ in appearance and function from their

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three-dimensional counterparts growing in a living body, it has been understood, however, that there are other factors influencing tissue growth dynamics. It is current opinion of the investigators that not only the internal stress is a critical factor but also the overall effect of the external environment must be regarded as a relevant and growing part of the analysis. In reality, an important reason cells are sensitive to growth conditions found in standard bioreactors is that fluid flow causes strong shear forces that discourage cell aggregation. This limits both the development of the tissue and the degree to which it possesses structures and functions similar to those found in the human body.

The perplexing difficulties that arise in the cultivation and subsequent analysis of living tissue samples stem from the greater complexity, lability and dynamic properties of these materials. These factors have all combined to limit investigations into the kinetics of the growth process.

Fortunately, the science of computational fluid dynamics and, in particular, related moving-boundary methods, are evolving rapidly, and are spreading from their traditional heartlands of physics, chemistry and mathematics into exciting new areas such as macromolecular crystal growth and modeling of molecular processes (e.g., Lappa, 2003a), and finally into the new field of tissue engineering and similar topics (see, e.g., Bahen et al., 2005).

Volume of Fluid methods (VOF, also known as volume tracking methods) and Level-set techniques have become popular in the last years as numerical techniques capable of modeling complex multi-phase flow separated by a moving interface as well for their capability to undertake a fixed-grid solution without resorting to mathematical manipulations and transformations (for a very comprehensive discussion dealing with the genesis and the evolution of these eulerian methods see, e.g., Lappa 2003b; 2004; 2005a,b; Esmaeeli 2005; Jimenez et al. 2005; Hogea et al. 2005 and Macklin and Lowengrub 2005).

In an earlier analysis (Lappa, 2003b) a new class of techniques, specifically devoted to tissue engineering and based on the fundamental principles at the basis of the aforementioned volume tracking methods, has been used to shed some light on the problem related to the determination of a mathematical formalism for the growth of cartilaginous tissue. It has been proven how these numerical methods can be used as an useful tool for the introduction of relevant surface kinetics.

The present analysis extends the earlier ones by providing a more rigorous presentation of the process leading to the determination of the aforementioned kinetics (illustrating in detail the heretofore unshown parametric investigation necessary for the determination of the exponent in the dependence that relates the tissue growth rate to the fluid-dynamic shear stress) and by using them to provide predictive numerical solutions for conditions which, even though very interesting from a theoretical point of view, have not been experimentally focused upon till date.

The case of two samples hydrodynamically interacting in a rotating bioreactor, in fact, is considered. The interplay between two specimens in a tandem arrangement is investigated in terms of the shear stress environment that occurs around the trailing scaffold due to the fluid-dynamic wake released by the leading one and in terms of the consequent construct morphological evolution.

The presence of two scaffolds is considered for a more realistic simulation of effective experimental conditions encountered during cartilaginous tissue growth by means of the rotating bioreactor technique; as shown by Freed et al. (1997) many scaffolds can ”hover” within the growth reactor in fixed equilibrium positions; tandem configurations are possible as well as groups of scaffolds staying together for a long time (Vunjak-Novakovic, 2006).

2 The rotating-wall perfused Bioreactor

Bioreactors are generally defined as devices in which biological and/or biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions (e.g., pH, temperature, pressure, nutrient supply and waste removal, see e.g., Yu et al., 2005).

In an effort to enhance three-dimensional tissue formation, recently scientists have developed a ground-based facility for cell and tissue culture called rotating-wall perfused vessel (RWPV). This instrument cultures cells in a slowly rotating horizontal cylinder with a coaxial tubular oxygenator, which produces lower stress levels on the growing cells than earlier Earth-based experimental environments.

In practice, it simulates the weightless environment of space on Earth by putting biological constructs in a growth medium that constantly rotates and keeps them in endless freefall. The rotation rate of the external vessel

can be adjusted, in fact, so as to suspend each tissue construct about a fixed position within the vessel as viewed by an external observer (Obradovic *et al.* (2000)). This condition guarantees dynamic equilibrium of the operative forces gravity, buoyancy and drag, while maintaining each tissue construct in a state of continuous freefall. In other words, a tissue construct falling through the culture media in the RWPV is fed as it effectively sieves through the nutrient media.

As mentioned before, a sample grown by the rotating vessel technology undergoes lower stress levels with respect to conventional reactors and thus can grow along three dimensions. However, since the tissue constructs actually hover within the bioreactor and are completely surrounded by moving liquid, the shear stress still plays an important role.

The next section briefly describes the experiments of Freed, *et al.* (1997) and Obradovic *et al.* (2000) that are considered here as a basis for the subsequent numerical/theoretical analysis.

3 The experiments

Functional cartilaginous constructs for scientific research and eventual tissue repair were cultivated by Freed *et al.* (1997) and Obradovic *et al.* (2000) in bioreactors starting from chondrocytes (cartilage cells) immobilized on polymeric scaffolds with diameter 5 mm and thickness 2 mm. Cell seeding of scaffolds – that is, the dissemination of isolated cells within a scaffold – is, in fact, the first step in establishing a 3D culture (see Radisic et al., 2006). These disklike structures mimic the body's internal environment. They gradually degrade as the cells regenerate a tissue matrix consisting of water (soft tissues consist in fact primarily of various cell types, an extracellular matrix-ECM, and abundant water) and other fundamental components; in the experiments of Freed *et al.* (1997) and Obradovic *et al.* (2000) the main cartilage components were glycosaminoglycan (GAG, $\rho_{GAG}= 6{\cdot}10^{-2}$ [g cm^{-3}]) and type II collagen ($\rho_{collagen}= 2.7{\cdot}10^{-2}$ [g cm^{-3}]). Growth was obtained by using a glucose nutrient solution ($C_{glucose(o)}$=$4.5{\cdot}10^{-3}$[g cm^{-3}]). Temperature was maintained at 37 [$^\circ$C]. The final construct shape after 30 days is shown in Fig.1 (the size of the construct increased at a constant rate of 70 [μm/day] = $8.1 \cdot 10^{-8}$[cm s^{-1}] = 8.1 [Å s^{-1}]).

Section 4 illustrates the philosophy of modeling together with the fundamental equations governing the fluid behavior, the tissue morphological evolution and the surface condition that "links" fluid motion effects to the effective tissue growth and vice versa (mutual "interplay" condition). Finally, section 5 is split into two parts. In the first part comparison between numerical and experimental results for a single specimen is used as a means to effectively define the functional dependencies (among them the exponent of the shear stress) in the aforementioned "interaction" boundary condition at the tissue surface; in the second part the complete model is used to simulate tissue growth for a double-scaffold configuration for which it is expected that the final shape of a construct may significantly depend on its relative position in the bioreactor.

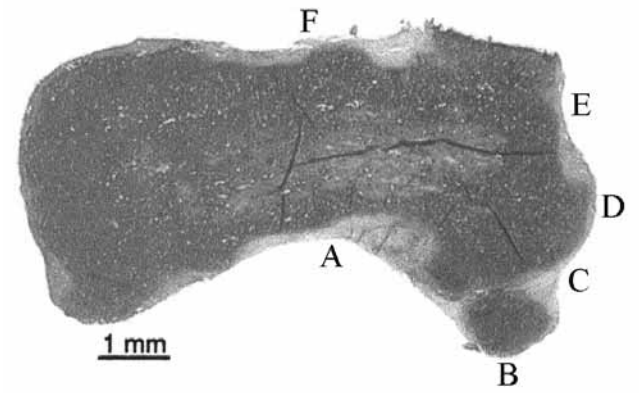

Figure 1 : Experimental histological cross-section of cartilage construct cultured for 30 days (Obradovic *et al.*, 2000. Reproduced with permission of the American Institute of Chemical Engineers. Copyright © 2000 AIChe. All rights reserved).

4 The mathematical model

4.1 The governing equations

The flow and the related distribution of nutrients in the bioreactor are governed by the continuity, Navier-Stokes and species equations, that in non-dimensional conservative form read :

$$\underline{\nabla} \cdot \underline{V} = 0 \tag{1}$$

$$\frac{\partial \underline{V}}{\partial t} = -\underline{\nabla} p - \underline{\nabla} \cdot [\underline{VV}] + S_c \nabla^2 V - S_c \frac{1}{\eta} \underline{V} \tag{2}$$

$$\frac{\partial C}{\partial t} = \left[-\underline{\nabla} \cdot (\underline{V} C) + \nabla^2 C \right] \tag{3}$$

where $S_c = \nu/D$ is the Schmidt number, (ν is the kinematic viscosity of the culture liquid, D is the diffusion coefficient of the nutrient solute). The non-dimensional form of the equations results from scaling the lengths by a reference distance (L= 1 [cm]), the time by L^2/D, velocity $\underline{V}$ and pressure p by D/L and $\rho_S D^2/L^2$ (ρ_S=density of the culture medium), respectively; the initial concentration of nutrient is denoted by $C_{(o)}$ ([g cm^{-3}]). The term $-S_c\underline{V}/\eta$ in equation (2) is the Darcy term added to the momentum equation to damp convection inside the tissue (further details about this term will be provided in section 4.3).

The presence of the solid mass (tissue) suspended inside the reactor is treated by means of a multi-phase methodology (OTGVOF: Volume of fraction method for Organic Tissue Growth). Such a technique is used to address the geometric complexity introduced by the tissue samples and their growth process. The organic solid mass stored in the generic computational cell is taken into account by assigning an appropriate value of ϕ (phase variable) to each mesh point (ϕ=1 biological tissue, ϕ=0 feeding solution and $0 < \phi < 1$ for an interfacial cell).

In practice, the key element for the OTGVOF method is its technique for adjourning ϕ. Upon changing phase, the ϕ-value of a computational cell is adjusted to account for mass absorption, this adjustment being reflected in the concentration distribution of nutrients in liquid phase as a sink. The modeling of these phenomena is based on the introduction of a group of differential equations, strictly related, from a mathematical point of view, to the kinetic conditions used to model mass absorption at the specimen surface (see the next section).

4.2 The surface boundary condition

The mass supply in the living tissues is possible through the biochemical interaction of the tissue with its environment. This means that the living tissue is an open system. Growth proceeds by the incorporation of growth units (atoms, molecules or small aggregates) from the feeding solution to the biological construct. Nutrients available in the culture medium are incorporated and converted into the main tissue components following a chain of biochemical transformations.

In principle the equation governing the aforementioned kinetics must take into account the main aspects of the growth behavior of biological tissues, i.e. the availability of nutrients, the slow surface absorption mechanisms, the effect of the surface shear stress and other environmental parameters:

$$D\frac{\partial C}{\partial n}\bigg|_i = \lambda \overline{f}(C_i, \tau, T) \tag{4a}$$

where $\partial C/\partial n$ is representative of the effective flux of nutrients absorbed at the tissue surface according to its intrinsic mechanisms of growth, $\overline{f}$ is a function depending on the type of tissue, C_i is the concentration of the nutrient at the construct/liquid interface; as anticipated, D is the diffusion coefficient of the nutrient in the feeding solution, T is the temperature of the culture medium, λ is a kinetic coefficient having the dimensions of a velocity (it is defined as a proportionality factor between the effective flux of nutrients and the right member of the equation that accounts for the surface kinetics) and τ is the fluid-dynamic shear stress at the sample/feeding-solution interface (*n* denotes direction perpendicular to the advancing tissue surface).

Since the temperature of the system was maintained constant (T=37 [$^\circ$C]) during the experiments of Freed *et al.* (1997) and Obradovic *et al.* (2000), hereafter it is no longer considered as an active variable affecting the growth kinetics:

$$\frac{\partial C}{\partial n}\bigg|_i = \frac{\lambda}{D}\overline{f}(C_i, \tau) \tag{4b}$$

At this stage, for the convenience of the reader who is not an expert in the field of tissue engineering, it should be emphasized that the reason why the flux is a function of C is not related to fluid motion around the tissue. Eq.(4) is not based on fluid dynamics arguments but on the intrinsic nature of the growth process. The tissue surface absorbs nutrients according to their concentration. The larger is C, the larger is the rate with which they are incorporated by the tissue and the tissue grows. This means that $\partial C/\partial n$ in eqs. (4) is not the slope of the concentration gradient at the tissue surface as it is determined by the fluid dynamics around the sample (i.e. by the effect of fluid motion on the concentration field); rather it is the effective flux of absorbed nutrients as it is determined by the growing tissue: it depends on the type of tissue (e.g., cartilage, heart, etc.), on the availability of nutrients (i.e. on "C") and on the "sensitivity" of the tissue surface to the effect of shear forces (exerted by the external environment).

4.3 Mass evolution equation

As explained before, the contribution $\left.\frac{\partial C}{\partial n}\right|_i$ represents the mass exchange flux between the solid and liquid phases (i.e. organic sample and feeding solution) driven by the tissue intrinsic mechanisms of growth. Consequently the mass M (tissue matrix coming from incorporation and conversion of nutrients) stored in computational cells that are undergoing phase change can be computed according to the equation:

$$\frac{\partial M}{\partial t} \propto \frac{\lambda}{D}\overline{f}(C_i,\tau)\Delta s \tag{5}$$

where Δs is the reconstructed portion of the tissue surface (by definition perpendicular to the interface normal vector $\hat{n}$) bounded by the frontier of the control volume (computational cell) located astride the tissue surface. The determination of this parameter requires an additional effort since the shape of the construct for a fixed time is not known a priori and must be determined as part of the solution (see Lappa, 2003b).

The non-dimensional volume of the tissue mass stored in a grid cell can be computed as:

$$\Delta v|_{stored} = \frac{1}{L^3}\frac{M}{\sum\limits_k \rho_k} \tag{6}$$

where ρ_k are the partial densities of the tissue main components (ρ_{GAG} and $\rho_{collagen}$ for the present case); correspondingly:$\phi = \frac{\Delta v|_{stored}}{\Delta v}$where Δv is the volume of the computational cell.

Therefore the phase field equation reads

$$\frac{\partial \phi}{\partial t} = 0, \quad \text{if } |\nabla\phi| = 0$$

$$\frac{\partial \phi}{\partial t} = \frac{\lambda\overline{f}(C_i,\tau)L\Delta s}{D\sum\limits_k \rho_k \Delta v}, \quad \text{if } |\underline{\nabla}\phi| \neq 0, 0<\phi<1 \tag{7}$$

with C satisfying eq. (4b).

Eqs. (4) and (7) behave as moving boundary conditions, their solution being strictly associated with the computational check on the value of ϕ and its gradient. These equations are the core of the OTGVOF computational method. Their solution of course must be coupled with the incompressible Navier-Stokes and species transport equations (1-3) which provide the velocity and concentration distribution in the liquid surrounding the biological construct.

The multiphase region (region where nutrients are absorbed and increase of mass occurs, $|\underline{\nabla}\phi| \neq 0$, $0<\phi<1$) is treated as a porous material characterized by an isotropic permeability η. In the present analysis permeability is assumed to vary according to the Carman-Kozeny equation:

$$\eta = \frac{(1-\phi+\varepsilon)^3}{(\phi+\varepsilon)^2}, \quad \varepsilon = 10^{-5} \tag{8}$$

See Lappa (2003b) for further details on the numerical method.

5 Numerical simulations.

5.1 Computational domain and basic assumptions

The generic specimen is supposed to maintain axisymmetric shape during the growth process, its symmetry axis being coincident with the symmetry axis of the cylindrical scaffold initially used to seed the chondrocytes. The velocity field is supposed to be uniform and parallel to the above-mentioned axis at a sufficient distance (about 1 [cm]) from the construct (undisturbed conditions). Its intensity there is assumed to be equal to the terminal velocity with which the scaffold falls through the bioreactor (endless sedimentation process).

A 20 [mm] wide (radius=10 [mm]) computational domain is simulated. Figures 2 show the geometry of the computational domain and the boundary conditions for the case of two scaffolds in a columnar arrangement(note that due to the axisymmetry of the model, for the sake of simplicity and brevity, only half of the generic meridian plane is shown therein): y=0 corresponds to the symmetry axis, y=1 to the distance from the axis at which undisturbed flow conditions prevail, x=0 corresponds to the "inflow" section (as explained in section 2, in the laboratory frame the construct holds a fixed position and the fluid moves upward in the direction opposite to the gravity force).

For y=0 "mirror" boundary conditions are imposed. i.e. the radial velocity component (v) is zero and the axial one (u) does not change across the axis (du/dy=0); for y=1 undisturbed flow conditions are imposed i.e. v=0 (the undisturbed flow does not have a radial velocity component) and u=U; for x=0 again undisturbed flow

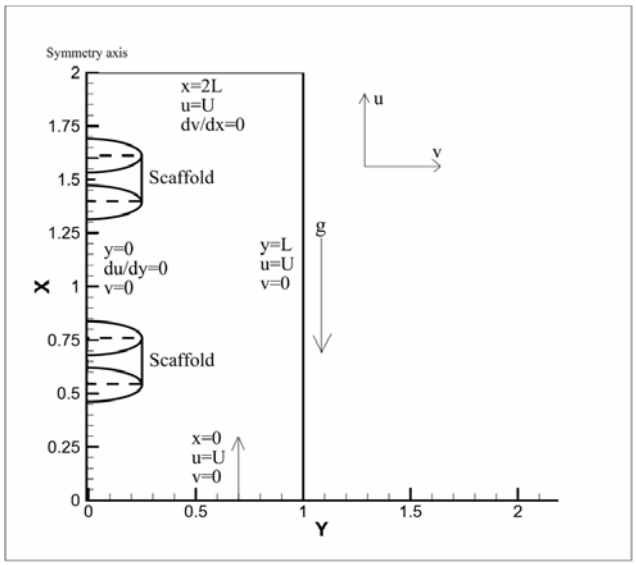

Figure 2 : Sketch of the configuration with boundary conditions and relative position of the scaffolds.

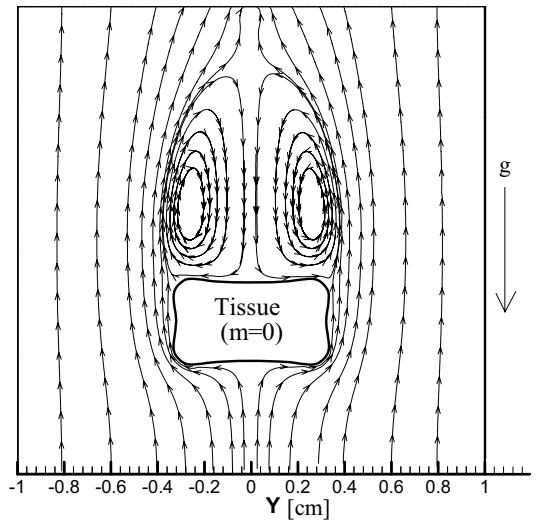

Figure 3 : Snapshot of growing tissue and surrounding velocity field after 30 [days], single scaffold, m=0.

conditions are imposed; in the outflow section the axial velocity component is assumed to be equal to the terminal velocity as for the inflow section (the volumetric rate of flow has to be constant throughout the system being the liquid an incompressible fluid).

Other data used for the computations are $D_{glucose}$=6.7·10^{-6} [cm^2 s^{-1}], kinematic viscosity of the liquid ν=8·10^{-3} [cm^2 s^{-1}]), U=4.64 [cm s^{-1}].

5.2 Single-scaffold case and determination of a mathematical formalism for the surface kinetics

During the real experiments the different effects summarized in the right member of eq. (4b) cannot be separated; the act all together and the related mutual interference can be very complex. This limitation, however, can be removed in the virtual environment provided by numerical simulation.

In such an environment, full understanding of the role played by each single factor (availability of nutrients and fluid-dynamic shear forces) and the relative importance with respect to each other in affecting the tissue growth process can be achieved by extensive parametric investigation. For instance, some parameters can be systematically taken into account while switching off the effect of the remaining ones.

Within this context, a first step is given by the application of eq. (4b) with no consideration for the effect of the shear stress. By analogy with the well-known case of macromolecular crystals (see, e.g., Pusey *et al.*, 1986) the surface kinetic condition can be written in non-dimensional form as :

$$\left.\frac{\partial C}{\partial n}\right|_i = \tilde{\lambda}(C_i) \tag{9}$$

where $\tilde{\lambda} = \lambda L/D$. The physical meaning of such a relationship is very simple: if C=0 (i.e. no nutrients are available), the sample does not grow; if the feeding solute is available, the growth rate (∂C/∂n) is proportional to its concentration at the interface through the kinetic coefficient dependent on the type of tissue.

Figure 3 shows the results obtained by application of eq. (9). In such simulations the necessary value for the kinetic coefficient (λ= 3·10^{-6} [cm s^{-1}]) has been obtained tuning its value in order to match the growth rate observed during the experiments (70 [μm/day]). These results clearly show that, even if the final size of the sample corresponds to the experimental one, its final shape does not reproduce the experimental result in Fig.1. The tissue maintains the initial shape related to the cylindrical scaffold used to seed the cartilage cells, i.e. it tends to grow equally in all directions.

Therefore the shear stress must be regarded as a significant parameter affecting tissue growth. The related effect is modeled here by the introduction of a function $F(\tilde{\tau})$

multiplying C in the second member of eq. (9), i.e.

$$\left.\frac{\partial C}{\partial n}\right|_i = \tilde{\lambda} F(\tilde{\tau})(C_i) \tag{10}$$

such a formulation, in fact, is a natural extension of eq. (9) and still gives no growth ($\partial C/\partial n=0$) in the case of no nutrients; $\tilde{\tau}$ is the non-dimensional fluid-dynamic shear stress at the tissue/liquid interface ($\tilde{\tau} = \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x}\right)$, u and v being the non-dimensional velocity components along x and y respectively, see Fig.2).

The presence of the term $F(\tilde{\tau})$ is necessary. It provides the required theoretical formalism for the heretofore controversial and poorly understood effect of the fluid-dynamic surface stress; according to eq. (10) the diffusion of momentum towards the tissue interface deeply influences the growth process; in other words there is a direct effect of the fluid-dynamic shear stress on the surface incorporation and conversion kinetics. It affects $\partial C/\partial n$, i.e. it alters the manner by which nutrients are incorporated into the tissue mass and the tissue grows. In practice, the function $F(\tilde{\tau})$ accounts for the physiologic response of the biological tissue to the surface stress distribution that acts modifying the mechanisms responsible for tissue enlargement and therefore the rate of absorption of nutrients. There is a notable difference with respect to the case of lifeless macromolecular crystals (Lappa, 2003a; Wakayama et al., 2005) where only solute transport and crystal intrinsic mechanisms of slow addition of molecules to the crystalline structure play a significant role (the term $\tilde{\tau}$ does not appear in the equation for the kinetics). Of course, in this case convective effects can also be present, but they do not enter directly the growth mechanisms. Rather they have an indirect effect on the concentration distribution around the growing crystal modifying the local values of C with respect to purely diffusive conditions (see, e.g., Lappa, 2003a).

With regard to the explicit dependency of F on $\tilde{\tau}$, it can be determined by parametric simulations. Such an analysis has initially shown that F does not behave as a linear function of $\tilde{\tau}$. The results obtained for F$\cong \tilde{\tau}$ are plotted in Fig. 4.

Direct comparison of Fig. 4 and Fig. 1 highlights that a linear dependence of F on $\tilde{\tau}$ leads to significant overestimation of the local growth rates close to lower corners of the construct. Such a deviation from the experimental results tends to become even larger when a quadratic dependence is assumed (F$\cong \tilde{\tau}^2$see Fig. 5).

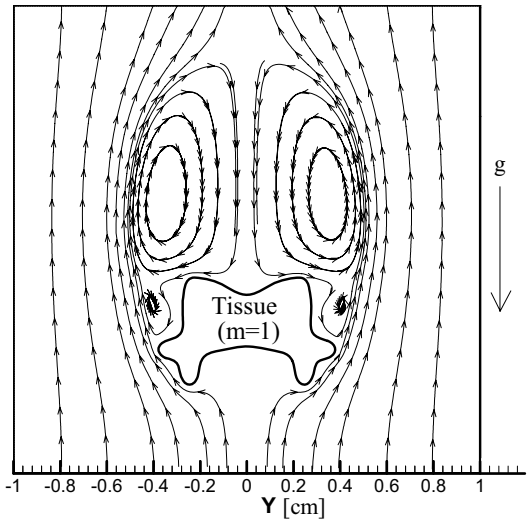

Figure 4 : Snapshot of growing tissue and surrounding velocity field after 30 [days], single scaffold, m=1.

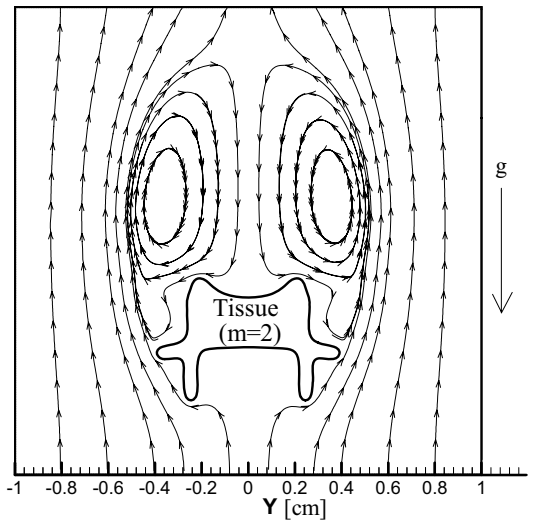

Figure 5 : Snapshot of growing tissue and surrounding velocity field after 30 [days], single scaffold, m=2.

For this reason the general form F$\propto \tilde{\tau}^m$ has been assumed and simulations have been carried out for different fractional values of $m < 1$ (1/2, 1/3, 1/4) up to match the behavior observed during the experiments.

This method has shown that the best possible agreement is achieved for m=1/2 or m=1/3 (F$\propto \tilde{\tau}^{1/2}$ or F$\propto \tilde{\tau}^{1/3}$ shown in Figs. 6 and 7, respectively).

Beyond the numerical results discussed before, there are several lines of experimental evidence in the literature that seem to support a role for shear stress in the transla-

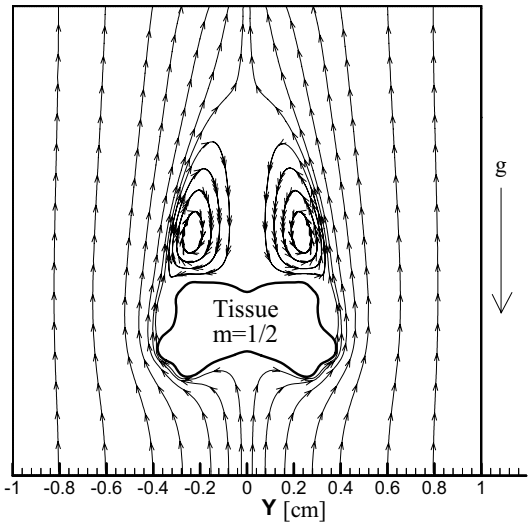

Figure 6 : Snapshot of growing tissue and surrounding velocity field after 30 [days], single scaffold, m=1/2.

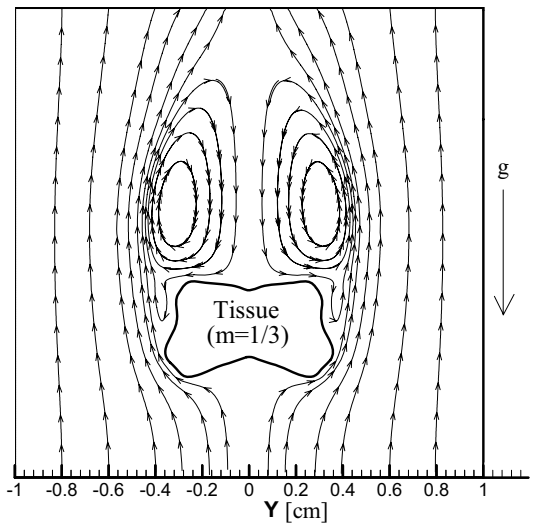

Figure 7 : Snapshot of growing tissue and surrounding velocity field after 30 [days], single scaffold, m=1/3.

tion of mechanical culture conditions into tissue effects for a variety of conditions and types of cells, see, e.g. the analyses of Garcia-Briones and Chalmers (1994), Shiragami and Unno (1994), Zhang et al. (1995), Joshi et al. (1996), Vunjak-Novakovic et al. (1998), Gregoriades et al. (2000), Chisti (2001), etc.

Along these lines, it is worth noting that recognition of the critical links between fluid-mechanics and tissue biochemistry and the related introduction of a mathematical formalism for different cell-types will lead to novel strategies for the development of new drugs and engineered tissues, as well as biomimetic microdevices and nanotechnologies that more effectively function within the context of living tissues. As a significant and recent example of such potential applications the reader may consider the study of Gemmiti and Guldberg (2006). A novel parallel-plate bioreactor has been specially designed to apply a desired level of fluid flow-induced shear stress to tissue-engineered articular cartilage in order to improve the matrix composition and mechanical properties.

These researchers have seeded primary bovine articular chondrocytes into the bioreactor at high densities (1.7×106 cell/cm^2) without a scaffold and cultured for two weeks under static, no-flow conditions. A mean fluid flow-induced shear stress of 1 dyne/cm^2 was then applied continuously for 3 days. The application of flow produced constructs with significantly higher amounts of collagen compared to static controls. Concurrently, the tensile Young's modulus and ultimate tissue strength were significantly increased in flow samples compared to static controls. These findings prove that controlled flow-induced shear stresses may be used as an effective functional tissue-engineering strategy for improving matrix composition and mechanical properties in vitro.

5.3 Two scaffolds in a tandem configuration

In this section the numerical model defined in the earlier sections on the basis of direct comparison with available experimental results is used to provide predictive practical information for the case of a two-scaffold arrangement (leading scaffold at x=0.6, trailing scaffold at x=1.5) for which the fluid-dynamic interplay between the two bodies is expected to alter the final construct shape with respect to the case of an isolated scaffold.

The sequence of evolution stages for m=1/3 is depicted in Figs. 8. Each figure of the sequence corresponds to a different snapshot of the growth process.

A toroidal roll is created behind each growing specimen. This behavior is due to the obstruction created in the fluid flow by the presence of the disk-shaped tissue with its circular area perpendicular to the direction of motion. The toroidal convection roll appears in the generic meridian plane in the form of two vortices located behind each body in the downstream direction. The two vortices are embedded in a low velocity region hereafter referred to

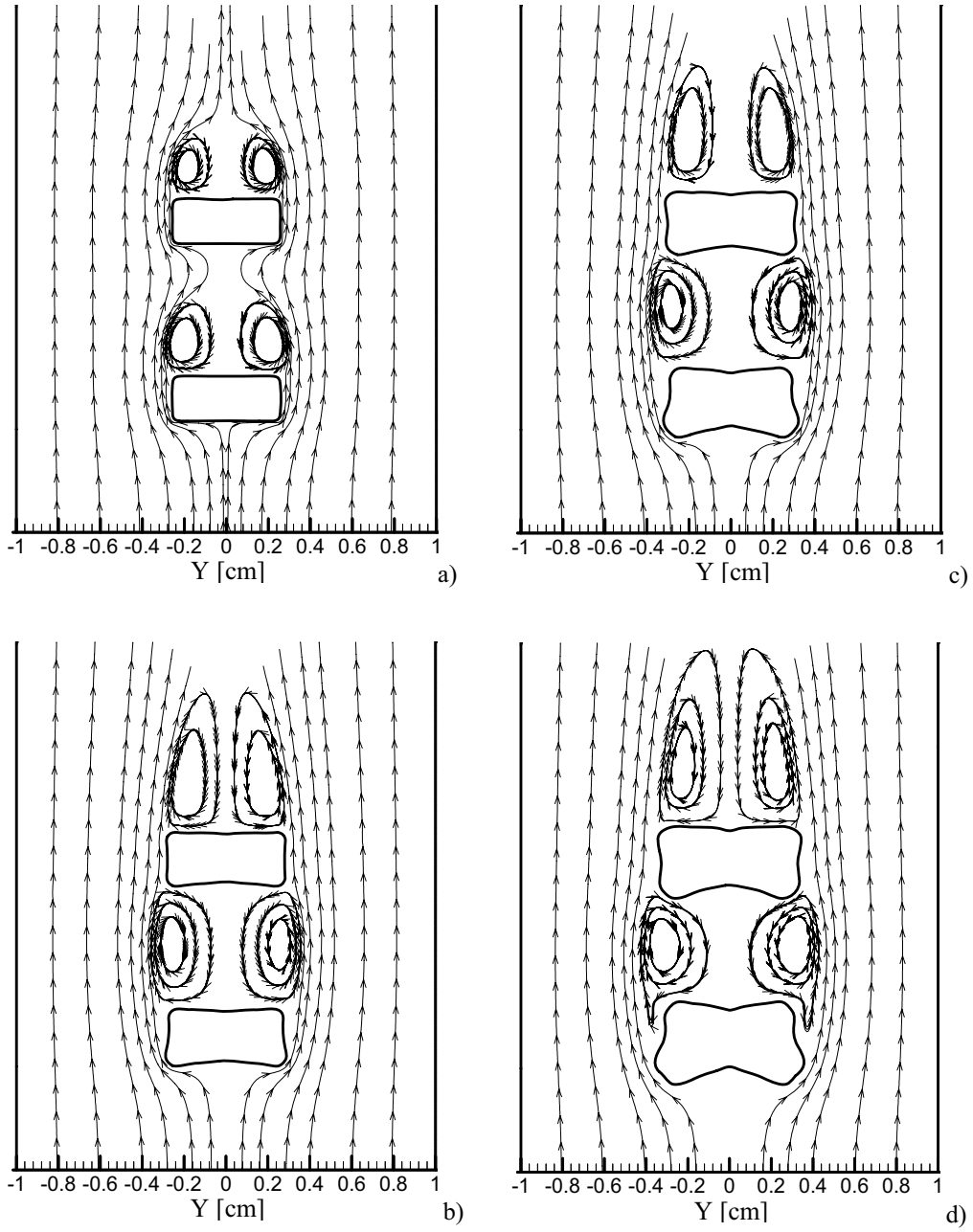

Figure 8 : Snapshots of growing tissue and surrounding velocity field (m=1/3): (a) after 1 [day], (b) after 10 [days], (c) after 20 [days], (d) after 30 [days].

as wake.

The interaction of the flow entering into the computational domain from the lower boundary with the leading sample leads to two main effects. One is the aforementioned creation of a wake, the second one is an effect of deceleration/acceleration of the fluid. The flow is strongly curved for $x < 0.5$ since it enters the computational domain directed along x and then it is forced to turn around the construct. Due to this path and its initial conditions, the fluid is initially decelerated along x up to the stagnation conditions on the tissue surface; then it is accelerated in the downstream direction due to the cross-sectional area reduction associated with the presence of the specimen; the acceleration, however, is considerably

damped when the fluid "feels" the presence of the second body.

This structure is crucial in determining the distribution of surface shear stress and thus the growth behavior for both the leading and trailing specimens.

First the attention is focused on the leading (lower with respect to the dynamical sedimentation process) body, then the effect related to the trailing (upper) one are depicted in detail.

Here, the simulations show that corners of the leading tissue grow faster than the analogous edges of the trailing one; this effect leads to morphological instability and to a macroscopic depression around the center of the faces like the case of a single isolated scaffold (as shown by Lappa, 2003b, a protuberance on the interface sees a higher shear stress and, according to eqs. (4) and (7), grows faster than a depression, which sees a lower shear stress).

According to Figs. 8, the depth of the face depressions is proportional to the size of the specimen, i.e. it increases during the growth. As time passes and the tissue widens, in fact the disturbance in the flow field produced by the presence of the construct becomes larger. This behavior in turn increases the value of the shear stresses responsible for the growth of protuberances on the surface (Lappa, 2003b).

Like the case of an isolated sample, the convection effect on the lower body results in higher local shear stresses near the surface where the flow is incoming (lower face of the leading sample) and in a lower shear stresses near the surface where the flow is outgoing (upper face). This behavior can be explained according to the fact that the upper side faces a region where recirculating flow occurs and therefore the fluid is close to stagnation conditions. For this reason the shear stresses causing growth there are weakened and correspondingly the growth rate is reduced. For the same reasons onset of morphological instabilities is prevented for the leading-sample upper side (the tissue surface facing the wake is almost plane and without irregularities).

In a similar way, onset of morphological instabilities is prevented for the upper side of the trailing construct.

For the second sample, however, the increase of size and the face growth rates do not show significant difference for the top and bottom sides. This occurs since in this case the incoming flow (wake from the lower sample) carries quasi-stagnant liquid. The trailing construct is bounded by its own wake by one side and by the wake of the lower specimen by the other side. The shear stresses in liquid phase are small regardless the flow is incoming or outgoing. This, on the other hand, explains why the size of the leading construct tends to be larger than that of the upper one (Fig. 9) and why face depressions do not occur for the latter (a depression is still present on the lower side but its extension is considerably reduced with respect to the depression that occurs on the lower side of the leading tissue). The upper sample is located in a quasi-stagnant liquid zone where the velocity is small and the related gradients are damped; this considerably weakens the morphological instabilities and related effects on the construct shape.

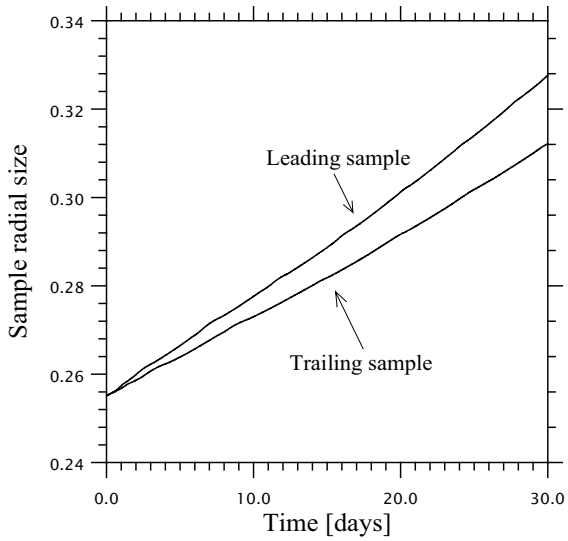

Figure 9 : Specimen maximum radial size ([cm]) as a function of time ([days]).

Analysis of evolution in time of the growth process in Figs. 8 shows that at the beginning (after few days) the wake of the lower scaffold does not directly interact with the upper scaffold. The wake axial extension is quite limited with respect to the distance between the two bodies. As time passes, however, it widens in both the axial and radial directions (see frames, (b), (c) and (d) in Fig. 8. According to this behavior, the difference between the size of the two samples tend to diverge as shown in Fig. 9.

6 Conclusions

A Volume of fraction method has been used to track the evolution of the solid/liquid interface of a single sample of living tissue surrounded by a nutrient solution focusing on the surface metabolism and its sensitivity to many local environmental factors.

Computer graphics has allowed to discern the effect and the intensity of each physicochemical parameter through direct visualization of the morphological evolution of the tissue shape, and comparison with experimental results. Precise mathematical laws have been introduced to model the surface growth kinetics in which the exponent of the fluid-dynamic shear stress is between 1/3 and 1/2 ($1/3 \leq m \leq 1/2$).

The numerical strategy defined on the basis of direct comparison with available experimental results then has been used to provide predictive practical information for the case of a two-scaffold arrangement for which the fluid-dynamic interplay between the two bodies is expected to alter the final construct shape with respect to the case of an isolated scaffold.

The face growth rates have been found to depend on the complex structure of the convective field and to be nonuniform across the sample face (growth rate always lower at the center than at the corner).

The extension of face depressions for the trailing specimen is considerably reduced with respect to the leading one since the upper sample is located in a quasi-stagnant liquid zone where the velocity is small and the related gradients (shear stresses) are damped.

The specimens interact mainly via the influence of the wake of the lower construct on the upper construct. The rising fluid coming from the lower body wraps around the upper one and has a major role in determining the velocity gradients on its surface. These gradients are weakened and for this reason the upper construct exhibits lower values of the growth rate and the onset of morphological instabilities is prevented.

The present analysis shows both the potential and challenges of fluid-dynamic mathematical modeling of *in vitro* organic tissue growth. The fundamentals equations and concepts herein presented are devoted to experts in the field of CFD and volume of fluid (VOF) methods. Nevertheless the work tries to create a bridge between the fluid mechanics and biotechnology communities under the optimistic idea that the contacts established between the two fields will develop into an ongoing, mutually beneficial dialogue.

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