The growth and the fluid-dynamics of protein crystals and soft organic tissues: models and simulations, similarities and differences

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Summary

The fluid-dynamic environment within typical growth reactors as well as the interaction of such flow with the intrinsic kinetics of the growth process are investigated in the frame of the new fields of protein crystal and tissue engineering. The paper uses available data to introduce a set of novel growth models. The surface conditions are coupled to the exchange mass flux at the specimen/culture-medium interface and lead to the introduction of a group of differential equations for the nutrient concentration around the sample and for the evolution of the construct mass displacement. These models take into account the sensitivity of the construct/liquid interface to the level of supersaturation in the case of macromolecular crystal growth and to the "direct" effect of the fluid-dynamic shear stress in the case of biological tissue growth. They then are used to show how the proposed surface kinetic laws can predict (through sophisticated numerical simulations) many of the known characteristics of protein crystals and biological tissues produced using well-known and widely-used reactors. This procedure provides validation of the models and associated numerical method and at the same time gives insights into the mechanisms of the phenomena. The onset of morphological instabilities is discussed and investigated in detail. The interplay between the increasing size of the sample and the structure of the convective field established inside the reactor is analyzed. It is shown that this interaction is essential in determining the time-evolution of the specimen shape. Analogies about growing macromolecular crystals and growing biological tissues are pointed out in terms of behaviours and cause-and-effect relationships. These aspects lead to a common source (in terms of original mathematical models, ideas and results) made available for the scientific community under the optimistic idea that the contacts established between the "two fields of engineering" will develop into an ongoing, mutually beneficial dialogue.

Key words: growth kinetics, fluid motion, mathematical models, moving boundary method, morphology evolution.

Running headline:

Organic engineering: models and simulations
1. Introduction

In the last years great interest has been directed towards crystals of biological macromolecules and towards the crystallization process of protein substances. In fact, single crystals with good diffraction properties and structural quality are needed to achieve high resolution data on protein structure. These data in turn are needed for industrial production of pharmaceuticals and devices. Because of the large size and complexity of protein molecules and the weakness of the bonding forces between them, the experimental principles and methodologies (and associated mathematical models) underlying high-quality inorganic crystal growth have been proved to be of little help in protein crystallization. The perplexing difficulties that arise in the crystallization of macromolecules in comparison with conventional small molecules stem from the greater complexity, lability and dynamic properties of proteins. Proteins are very sensitive to their environment and if exposed to sufficiently severe conditions may denature and/or degrade. They must be constantly maintained in a thoroughly hydrated state at or near physiological pH and temperature. Thus common methods for the crystallization of conventional molecules such as evaporation of solvent, dramatic temperature variation, or addition of strong organic solvents are unsuitable and destructive (McPherson (1990), Kuznetsov, Malkin, Greenwood and McPherson (1995)). They have been supplanted with more gentle and restricted techniques. Since proteins are sensitive macromolecules that readily loose their native structures, the only conditions that can support crystal growth are those that cause little or no perturbation of the molecular properties. For these reasons the protein crystals are grown from a solution to which they are tolerant. This is called the mother liquor. Because complete hydration is essential for the maintenance of structure, protein crystals are always bathed in the mother liquor. The mother liquor in turn is usually contained in a proper environment, specifically designed to favour growth, called the "protein reactor". The protein reactor is a chamber where the strategy employed to bring about crystallization is to guide the system in a very gentle manner and "very slowly" toward a state of reduced solubility by modifying the properties of the solvent.
In the light of the above arguments, "Protein Crystal Engineering" can be defined as the field that studies the way to achieve the best possible conditions for the crystallization of macromolecular substances needed for progress in biotechnology, pharmaceutics and drug.

On the other hand, given our current expertise in growing organic tissues as model systems for controlled studies of tissue development the concept of "Tissue Engineering" is also nearing reality. Tissue engineering is a new field that enables tissue equivalents to be created from isolated cells in combination with biomaterials and "bioreactor culture vessels".

It provides a starting point for understanding the nature of tissue growth under real and idealized conditions. The selection of tissue replacement, the mechanisms affecting cell function and growth, the effects of environmental factors (such as gravity) on the body, cancer treatment and bio-product production are some examples of questions that can eventually be addressed by culturing functional tissue in bioreactors. In future moreover the constructs obtained by these techniques will be used for treatment of damaged human tissues and/or healthy organs. Tissue Engineering, in fact, has also been defined as "The use of naturally occurring and/or synthetic materials in conjunction with cells to create biologic substitutes to serve as functional tissue replacements".

Bioreactors are devices employed to culture tissues as diverse as human cancers and cartilage for both research and implantation purposes. The tissue to be cultured is placed within the reactor, which includes a means for supplying (liquid) "culture medium" to the growing tissue, providing it with nutrients and simultaneously removing waste products from its vicinity.

Multiple types of reactors exist, including perfusion vessels as well as spinner flasks, however the best environment has been proved to be the NASA "rotating vessel".

The attention of the Researchers in the last years has been focused in particular on the so-called "Rotary Culture System" since this technique has proven to be the most practical solution to provide a suitable culture environment supporting 3-D tissue assemblies.

The rotating bioreactor is a horizontally rotating cylinder completely filled with fluid culture medium. Under these conditions, the fluid rotates at the same rate as its container (the cylinder),
resulting in continuous suspension of the biological particles. The conditions in the bioreactor are very conducive to engineering tissues from individual cells and for simulating microgravity. It allows researchers to produce breakthrough results by creating three-dimensional cell cultures which are much more similar to tissues found in the human body (even if one of the major limitations to current tissue engineering is still the inability to vascularize the tissue in any manner).

The rotating bioreactor has applications on the ground as a simulator of microgravity i.e. it provides some of the physical operating conditions commonly associated with microgravity such as randomization of the gravity vector and allowing cells to fall at terminal velocity. Production of larger specimens however requires true microgravity. On Earth, samples may become so large (about 1 [cm]) that they are no longer suspended. In long space missions, large-sample growth in bioreactors have been studied in order to understand the extended growth (Freed, Langer, Martin, Pellis, Vunjak-Novakovic (1997)).

These studies have shown that the final size and shape of the specimens can be very sensitive to the different fluid-dynamic environments that occur inside the tissue-reactor according to whether the growth process is carried out on Earth or under microgravity conditions. For instance more spherical tissue samples have been obtained in space by Freed et al. (1997) with respect to ground-based experiments.

The use of microgravity has proven to affect also the growth of macromolecular protein crystals. Crystallization experiments carried out in microgravity conditions, in fact, have yielded protein crystals that resulted in diffraction data of significantly higher resolution than the best crystals of these proteins grown on Earth. Since an obvious difference between the space and Earth based experiments is the magnitude of the buoyancy forces in the solution, the role of solutal convection in protein crystal growth has been proved to be very important.

In the light of the above arguments some conclusions can be drawn about these topics. Both protein (crystal) engineering and tissue engineering deal with the growth of organic constructs in proper environments by suitable and gentle techniques. The "key factor" of these fields of engineering
consists in how to define the properties of the artificial environments where protein crystals or biological tissues respectively have to be grown in order to achieve the best possible conditions.

In particular, what turns out from the studies carried out under microgravity conditions for both cases of protein crystals and biological tissues is that the characters of the final samples change depending on the fluid-dynamic conditions under which they are operated. Hence, an understanding of the transfer of both momentum and mass within such devices as well as of the interplay of such flow with the intrinsic kinetics of the growth (still poorly understood) is crucial for further improvements in the fields of protein crystal and tissue engineering.

It is worthwhile to stress that despite the different fluid-dynamics of the growth processes (natural convection induced by the buoyancy in the case of protein reactors and "forced" convection due to dynamic construct settling in the case of rotating vessels) the phenomena exhibit notable analogies.

The present manuscript forms a preliminary but quite exhaustive attempt to shed some light on biological tissue growth phenomena from an organic crystal growers point of view. Moreover novel mathematical models are provided for both the topics. They deal with the surface kinetics of the growth process and are discussed in the same frame of "moving boundary methods" and mathematical models for growing specimens. Regardless of the nature of the growing sample (organic protein crystal or biological tissue) some common features are pointed out. This structured and systematic analysis leads to a common source (in terms of original mathematical models, ideas and results) made available for the scientific community under the optimistic idea that the contacts established between the "two fields" in the present manuscript will develop into an ongoing, mutually beneficial dialogue.

2. Surface kinetics

The growth of protein crystals shares many features with the case of biological tissue growth:

1) For both cases, on a molecular level, growth follows from the successive "addition/incorporation" of ‘growth units’ or ‘building blocks’ to a lattice. Growth units can consist of single molecules or possibly of clusters of molecules. In solution, growth units are
typically solvated i.e. are surrounded by highly regularly arranged shells of solvent molecules that interact with the growth unit in a bond-like fashion. In the case of macromolecular crystals the growth units are merely "added" to the crystal surface (the molecules orient and attach themselves to the growing surface) without changing their initial composition. In the case of biological tissues of course the mechanism is more complex. The growth units are incorporated and "converted" into the main tissue components. Consequently tissue enlargement occurs due to "internal cell division" and "production of extracellular matrix" supported by the aforementioned incorporation of nutrients in the biological cells.

2) Protein crystals and organic tissue specimens consist of:
(a) organic molecules forming a network; (b) ‘bound water’ that lines the large channels formed by the wide-open organic material network in bond-like arrangements; (c)’bulk water’.

Protein crystals, in fact, have widely open structures and incorporate up to 90% by volume of solvent within the network of protein molecules. Macromolecular crystals are mainly composed of solvent. The protein occupies the remaining volume so that the entire crystal is in many ways an ordered gel with extensive interstitial spaces through which solvent and other ‘small’ molecules may freely diffuse (see e.g. Rosenberger (1986) and McPherson (1990)).

In the case of biological tissues, as cells replicate, they “self associate” to form a complex matrix of collagens, proteins, fibers, and other chemicals. Also in this case water is the major component of the construct. Soft tissues consist in fact primarily of various cell types, an extracellular matrix, and abundant water (see e.g. Humphrey et al. (2001)).

3) The protein crystal and the organic tissue grow in ‘feeding solutions’ of known (initial) concentration.

4) The presence of a distinct boundary layer about the growing organic material (due to the depletion of the feeding substance in liquid phase) supports (for both the cases of protein crystal and organic tissue growth) the idea of competitive transport and surface growth kinetics in limiting the growth rate of the process.
These complicating features make it very unlikely that protein growth kinetics will follow any unmodified inorganic growth models. Hence not surprisingly, the few available results for protein growth rate dependence on bulk supersaturation deviate in form as well as magnitude considerably from any inorganic model predictions.

The ‘attachment rates’ in protein crystallization are considerably lower than in most inorganic systems (this slow interface kinetics has been interpreted in terms of the low symmetry and the small binding energies involved in protein crystallization). The growth kinetics are very slow also for the case of biological tissues (e.g. in the experiments of Freed et al. (1997) many months were required to complete the growth process).

Because of these theoretical difficulties, Pusey et al. (1986) expressed the growth rate of a protein crystal as a function of supersaturation introducing a ‘kinetic coefficient’ ([cm s⁻¹]) dependent on the physical properties of the protein.

This model is adopted in the present paper and further improved in order to extend it to the case of biological tissue growth.

Anisotropic growth is taken into account. This situation may occur for crystalline proteins which have high anisotropy (preferred orientations) in either their surface energy or atomic attachment kinetics and for biological tissues that exhibit different growth behaviour according to the orientation of the fibers with which they are composed.

In the case of protein crystals, "surface attachment kinetics" at the crystal surface depend on the local value of protein solubility and on a coefficient \( \lambda \) (kinetic coefficient) whose value may be different according to the local orientation of the crystal surface (surface-orientation-dependent growth, Coriell, Chernov, Murray, McFadden (1998)); using mass balance (see e.g. Rosenberger (1986)), and assuming a linear dependence of the growth rate by the interface supersaturation (see e.g. Lin et al. (1995) and Lappa (2003)), one obtains:

\[
\left( \frac{D}{\rho_p - \rho_C C_i / \rho_S} \right) \frac{\partial C_i}{\partial n} = \lambda \left( \frac{C_i - S}{S - \delta_o} \right)
\]
where $C_i$ is the concentration of the protein (solute) at the crystal surface, $D$ is the related diffusion coefficient in the mother liquor, $S$ is the solubility (its value is a function of the local concentration of a precipitant agent and/or of temperature), $\rho_p$ and $\rho_c$ are the protein mass density and the total mass density in the crystal, $\rho_s$ is the total density of the solution (mother liquor), $\delta_o$ is the width of the supersaturation zone in which no growth occurs, $\lambda(n)\hat{n}$ is the kinetic coefficient and $\hat{n}$ is the unit vector perpendicular to the crystal surface pointing into the liquid. The parameter $\delta_o$ takes into account the so-called ‘dead zone’, that according to many investigators, in the case of organic crystals, is due to the absorption of impurities that lead to strong retardation of the growth kinetics of the crystal. In the case of protein substances, such ‘impurities’ are not necessarily extrinsic contaminants, being often the result of protein ‘microheterogeneity’ (Monaco and Rosenberger (1993)).

Whenever protein in solute phase and solid crystal co-exist in equilibrium (saturation condition):

$$C_i = S$$

(2)

In a saturated solution, two states exist in equilibrium, the solid phase, and one consisting of molecules free in solution. At saturation, no net increase in the proportion of solid phase can accrue since it would be counterbalanced by an equivalent dissolution. Thus ‘crystals do not grow from a saturated solution’. The system must be in a non-equilibrium, or supersaturated state to provide the thermodynamic driving force for crystallization (McPherson (1990)). Solution must by some means be transformed or brought into the supersaturation state whereby its return to equilibrium, forces exclusion of solute molecules into the solid state, the crystal. As long as $C_i < S$, more solid material will dissolve if any. If, on the other hand, $C_i > S$, material will condense on any material already existing and augment its size. The ‘growth regime’ may be very complex and non-linear (see e.g. Vekilov, Iwan, Alexander and Rosenberger (1996)). Its features depend on several parameters and in particular it is a function of the ratio between surface-attachment kinetics (modelled by eq.(1)) and mass transport in liquid phase (diffusive or convective).
The strategy employed to bring about crystallization is to guide the system very slowly toward a state of reduced solubility by modifying the properties of the solvent.

The classical procedure for inducing proteins to separate from solution and produce a solid phase is to gradually increase the level of saturation of a precipitant agent.

Protein precipitants fall into four broad categories: (a) salts; (b) organic solvents; (c) long chain polymers and (d) low-molecular-mass polymers and non-volatile organic compounds.

The nucleation of new material is something else and the features of this phenomenon are out the scope of the present work (for theoretical analysis of this phenomenon see the excellent paper of McPherson (1990); for numerical models dealing with the phenomena of protein nucleation see e.g. Lappa, Castagnolo and Carotenuto (2002) and Lappa, Piccolo and Carotenuto (2003)); the term ‘precipitation’ refers in fact to the composite phenomenon of nucleation and subsequent growth.

Growth can take place at concentrations lower that those needed for nucleation, as long as \( C_i > S \). The solution is said to be *supersaturated* when the solute content is greater than S, and the degree of supersaturation \( \sigma = C_i / S \).

By analogy with these models, in the case of biological tissues it is reasonable to assume that the concentration of nutrient at the construct surface must satisfy a condition such as:

\[
D \left( \frac{\partial C}{\partial n} \right)_i = \lambda \left( \hat{\mathbf{n}} \right) C_i
\]

where \( C_i \) is the concentration of the nutrient (in the experiments of Freed et al. (1997) it is glucose) at the construct/liquid interface and D is the related diffusion coefficient in the feeding solution.

In the case of biological tissue growth, the role played in the case of organic crystals by the degree of supersaturation has to be replaced by the local value of concentration of nutrient in the culture medium.

If \( C_i = 0 \), in fact, no net increase in the proportion of solid phase can accrue since nutrients are not available (the tissue does not grow). On the contrary, if \( C_i > 0 \), tissue growth occurs and the rapidity of the phenomena is driven by the value of the kinetic coefficient \( \lambda \).
Note that the above model, introduced in the present paper by analogy with models for organic crystal growth, ignores the fact that, according to biomechanical growth laws proposed in the case of soft tissues, the rate of growth has to depend on the stresses.

According to many investigators (see e.g. Fung (1990), Rodriguez, Hoger, McCulloch (1994), Taber (1998a), Taber (1998b), Taber, Chabert, Varahoor and Perucchio (2001), Taber, Chabert (2002), Van Dyke and Hoger (2001)), growth and remodelling in tissues may be modulated by mechanical factors such as internal stress. A general continuum formulation for finite volumetric growth in soft elastic tissues based on their internal "mechanical state" therefore has been proposed. This dependence was introduced through the so-called "growth law", which is a constitutive equation for the rate of change of the growth tensor, and describes its dependence on mechanical quantities such as stress, strain, and strain energy. The growth tensor is determined from the growth law. Two excellent implementations of this theory have been used to model continuous growth of soft tissues. The first, developed by Taber and co-workers (Taber (1998a), Taber (1998b), Taber et al. (2001), Taber and Chabert (2002)), has been used to investigate the growth of the heart, arteries, and skeletal muscles. In this approach, the growth law was defined on an initial fixed reference configuration for the entire growth process. In the second method, proposed by Hoger and co-workers (Rodriguez et al. (1994), Van Dyke and Hoger (2001)), the growth model was defined on the current configuration of the loaded, growing material. This method was used to elucidate how residual stress arises during growth and how in turn the growth is affected by stress in the tissue.

How fluid shear stresses generated by blood flow in the vasculature can profoundly influence the phenotype of the endothelium by regulating the activity of certain flow-sensitive proteins (enzymes, for example) as well as by modulating gene expression was also shown from an experimental point of view by Topper and Gimbrone, (1999).

To model these aspects the kinetic condition for soft tissues is re-written in the present paper as:

\[ D \frac{\partial C}{\partial n} = \lambda \left( a \tau \right)^{1/2} C_i \]  \hspace{1cm} (4)
where a is a constant and $\tau$ is the fluid-dynamic shear stress at the tissue/liquid interface.

This condition finally takes into account the main aspects of the growth behaviour for biological tissues, i.e. the availability of nutrients ($C_l$), the slow surface kinetics ($\lambda$) and the effect of surface shear stress ($\tau$). It is a novel contribution to tissue engineering models.

The selection of the exponent (hereafter referred to as "e") for the shear stress in eq. (4) has been based on simple theoretical arguments. Since it is expected that the shear stress (i.e. transfer of momentum) acts modifying the incorporation and conversion kinetics (i.e. the manner with which nutrient is incorporated in the tissue and converted in its main components) and/or the "internal cell division" and "addition of extracellular matrix" mechanisms responsible for tissue enlargement, the phenomena have to depend on the diffusion of momentum towards the tissue surface whose propagation is governed by the square root of viscosity.

The choice of $e=1/2$ has also been validated by a parametric investigation: several simulations of the experiments of Freed et al. (1997) have been carried out for different values of the exponent, $e=n$ and $e=1/n$ ($n=1,2,3,4,$ etc.). This method has confirmed that $e=1/n$, $n=2$ (other values do not reproduce the experiments). The parametric investigation is not shown in detail for the sake of brevity.

3. Mathematical models and numerical technique

3.1 The moving boundary problem

From a numerical point of view, strictly speaking, the growing specimen (protein crystal or biological tissue) gives rise to a moving boundary problem. Moving boundary problems are still a challenging task for numerical simulation, have instigated much research, and have led to many different solutions.

The numerical simulations of these problems require a discretization or nodalization to allow numerical treatment on computers. There are two fundamentally different approaches: Eulerian methods use a frame of reference (discretization grid or mesh, control volumes, etc.) fixed in space,
and matter moving through this frame of reference. Lagrangian methods instead use a frame of reference (marker particles) fixed to and moving with the matter.

The first method capable of modelling multi-phase flow, separated by a moving interface, was the Marker and Cell (MAC) of Harlow and Welch (1965). This was in fact a combination of an Eulerian solution of the basic flow field, with Lagrangian marker particles attached to one phase to distinguish it from the other phase. While the staggered mesh layout and other features of MAC have become a model for many other Eulerian codes, the marker particles proved to be computationally too expensive and have been rarely used.

In the specific case of growth of macromolecular crystals and/or biological tissues from feeding solutions and in order to introduce novel numerical techniques, one must generally accomplish at least two things simultaneously: (a) determine the concentration field of nutrients in the liquid phase ("mother liquor" for protein crystals, "culture medium" for organic tissues) and (b) determine the position of the interface between the sample and the liquid phase. According to the technique used to address (a) and (b), in principle the numerical procedures able to solve these problems can be divided into two groups:

1) Multiple region solutions utilizing independent equations for each phase and coupling them with appropriate boundary conditions at the construct/liquid interface. This approach to the problem takes the point of view that the interface separating the bulk phases is a mathematical boundary of zero thickness where interfacial conditions are applied. These interfacial conditions couple to the concentration equations in the bulk and this system of equations and boundary conditions provides a means to address (a) and (b). Difficulties arise when this technique is employed since in this case in the vicinity of the growing construct front (phase change), conditions on mass flux and velocity evolution have to be accounted for. This effectively rules out the application of a fixed-grid numerical solution, as deforming grids or transformed co-ordinate systems are required to account for the position of the solid surface (see e.g. Noh, Koh and Kang (1998)).
2) Single region (continuum) formulations (or ‘phase field’ models) which eliminate the need for separate equations in each phase, by establishing conservation equations which are universally valid. From a theoretical point of view the major advantage of the single region formulations is that they do not require the use of quasi-steady approximations, numerical remeshing and co-ordinate mapping.

In a phase-field model, a phase-field variable $\phi$ which varies in space and time is introduced to characterize the phase of the material. In place of the ‘sharp’ transition from organic samples to feeding liquid that would characterize the multiple region formulations, here the phase-field varies smoothly but rapidly through an interfacial region. Additionally, in place of the interfacial jump conditions used in the multiple description, a differential equation applied over the entire computational domain governs the evolution of $\phi$. The effect is a formulation of the free boundary problem that does not require the explicit application of interfacial conditions at the unknown location of a phase boundary. For this reason in the present paper this strategy is adopted.

The first method capable of modelling complex multi-phase flow separated by a moving interface and capable to undertake a fixed-grid solution without resorting to mathematical manipulations and transformations, was the aforementioned Marker and Cell (MAC) of Harlow and Welch (1965). Instead of MAC, however, Volume of Fluid methods (VOF) and Level-Set methods have became popular in the last years (Hirt and Nichols (1981), Osher and Sethian (1988), Brackbill, Kothe and Zemach (1992), Gueyffier, Li, Nadim, Scardovelli and Zaleski (1999)). In particular, they have been used for the simulation of typical problems associated to gas/liquid or liquid/liquid systems. On the other hand, the ‘enthalpy method’ (Voller and Prakash (1987), Lappa and Savino (2002)) has been successfully applied to the case of thermal phase change problems characterized by the presence of moving solid/melt interfaces associated to the heating or the cooling of the system under investigation.
However, a complete numerical method aimed at the (‘moving boundary’) simulation of the growth of biological constructs in the frame of the new fields of protein and tissue engineering is still missing.

3.2 OGVOF - The Organic Growth Volume of Fraction Method

The OGVOF method, which, similarly to VOF and enthalpy methods, is a single region formulation, allows a fixed-grid solution to be undertaken and is therefore able to utilize standard solution procedures for the fluid flow and species equations directly, without resorting to mathematical manipulations and transformations.

This method accounts for the organic solid mass stored in the generic computational cell by assigning an appropriate value of $\phi$ to each mesh point ($\phi=1$ organic crystal or biological tissue, $\phi=0$ mother liquor or culture medium respectively and $0<\phi<1$ for an interfacial cell). The key element for the OGVOF method is its technique for adjoining $\phi$. Upon changing phase, the $\phi$-value of the 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 modelling of these phenomena leads to the introduction of a group of differential equations, strictly related, from a mathematical point of view, to the ‘kinetic conditions’ used to model mass transfer at the organic specimen surface. This technique and the associated mathematics represent an original contribution provided by the present analysis.

3.3 phase field equation

At the surface of the sample ($|\nabla \phi| \neq 0, 0<\phi<1$), nutrient concentration must satisfy the kinetic condition that in non-dimensional form reads:

$$\left.\frac{\partial C}{\partial n}\right| = f(C_i)$$  \hspace{1cm} (5)

where

$$f(C_i) = \tilde{\lambda}(\rho_p - \rho_v C_i / \rho_s)(C_i / S - 1 - \delta_o)$$ \hspace{1cm} (6a)

$$f(C_i) = \tilde{\lambda}(a \bar{\tau})^{1/2}(C_i)$$ \hspace{1cm} (6b)
with (6a) in the case of protein crystals, (6b) in the case of biological tissue respectively and
\[ \tilde{\lambda} = \lambda L / D, \] 
L being a reference length.

In eq. (5) the concentration gradient can be computed as:
\[ \frac{\partial C}{\partial n} = \nabla C \cdot \hat{n} \] 
(7)

where \( \hat{n} \) is the unit vector perpendicular to the construct/liquid interface pointing into the liquid phase:
\[ \hat{n} = -\frac{\nabla \phi}{|\nabla \phi|} = (\alpha, \beta) \] 
(8)

\[ \alpha = -\frac{\partial \phi}{\partial x} \sqrt{\left(\frac{\partial \phi}{\partial x}\right)^2 + \left(\frac{\partial \phi}{\partial y}\right)^2}, \quad \beta = -\frac{\partial \phi}{\partial y} \sqrt{\left(\frac{\partial \phi}{\partial x}\right)^2 + \left(\frac{\partial \phi}{\partial y}\right)^2} \] 
(9)

since \( \frac{\partial C}{\partial n} = \alpha \frac{\partial C}{\partial x} + \beta \frac{\partial C}{\partial y} \), (hereafter the subscript ‘i’="interface" is omitted) equation (5) can be re-written as:
\[ \alpha \frac{\partial C}{\partial x} + \beta \frac{\partial C}{\partial y} = f(C) \quad \text{if } 0 < \phi < 1 \] 
(10)

Eq. (10) with (6a) and (6b) for the case of protein crystals and soft tissues respectively, is used for the computation of the concentration of nutrients in solution at the solid/culture-medium interface.

This concentration in fact is not known a priori. In the light of the arguments pointed out in the previous sections, it changes dynamically during the growth process. Moreover each point of the interface tends to be characterized by a different value of this concentration according to the local level of supersaturation in the case of protein crystals or according to the local shear stress in the case of biological tissue.

The solution of eq. (10) requires a discretization or "nodalization" to allow numerical treatment on computers. The concentration gradients along x and y (\( \partial C/\partial x \) and \( \partial C/\partial y \)) are "discretized" by a finite difference technique i.e. they are expressed as "finite differences" between \( C \) at the surface (the unknown quantity in eq. (10)) and "external" nodal values lying in the liquid (these values are computed according to the transport species equations, see the next section). Therefore, according
to eqs. (10) and (6), C is determined as a function of "nodal" values of concentration in the liquid close to the considered point of the interface and of local supersaturation (protein crystal) or of local shear stress (biological tissue). Depending on the interface’s orientation, concentration gradients "across" the tissue surface are discretized by forward or backward schemes. These gradients read (note that the i and j subscripts indicate the relative position along x and y respectively of the "nodal" values of C):

\[ \frac{\partial C}{\partial x} = \begin{cases} \frac{C_{i+1,j}-C_{i,j}}{\Delta x} & \text{if } \alpha > 0, \\ \frac{C_{i,j}-C_{i-1,j}}{\Delta x} & \text{if } \alpha < 0, \end{cases} \]

\[ \frac{\partial C}{\partial y} = \begin{cases} \frac{C_{i,j+1}-C_{i,j}}{\Delta y} & \text{if } \beta > 0, \\ \frac{C_{i,j}-C_{i,j-1}}{\Delta y} & \text{if } \beta < 0. \end{cases} \]

After the computations of C at the solid/liquid interface \((C_{i,j})\), the term \(f(C_{i,j})\) can be seen as the local mass exchange flux between solid and liquid phase (i.e. crystal or tissue and feeding solution). The mass (construct mass coming from incorporation and/or conversion of nutrients) stored in computational cells that are undergoing phase change is governed by the equation:

\[ \frac{\partial M}{\partial t} = L^3 f(C) \, ds \quad (11) \]

note that in this equation M and C are dimensional whereas the other quantities have been posed in non-dimensional form (see section 3.4 for further details on the non-dimensional form of the equations). The term \(ds\) is the ‘reconstructed’ portion of the sample surface (by definition perpendicular to the interface normal vector \(\hat{n}\)) ‘bounded’ by the frontier of the control volume (computational cell) located astride the solid/liquid interface.

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

\[ dv \big|_{\text{stored}} = \frac{1}{L^3} \frac{M}{\rho_P} \quad (12) \]

where \(\rho_P\) is the protein mass density in the crystal in the case of macromolecular crystal growth and the sum of the densities of the tissue main components in the construct (partial densities) in the case of tissue growth process;

Correspondingly:

\[ \phi = \frac{dv \big|_{\text{stored}}}{dv} \quad (13) \]

where dv is the volume of the computational cell.
Therefore the phase field equation reads

\[
\frac{\partial \phi}{\partial t} = 0, \text{ if } |\nabla \phi| = 0
\]

\[
\frac{\partial \phi}{\partial t} = \frac{f(C) ds}{\rho_p dv}, \text{ if } |\nabla \phi| \neq 0, \ 0 < \phi < 1
\] (14)

with C satisfying eq. (10).

Equations (10), (11) and (14) behave as ‘moving boundary conditions’, their solution being strictly associated to the computational check on the value of \( \phi \) and its gradient.

According to equations (10) and (14), in the case of protein crystal growth, if the protein concentration is locally depleted, correspondingly, the solid mass stored in the computational cell grows and the phase variable is increased; on the other hand if mass stored in the cell begins to re-dissolve, protein is released in solute phase and the local value of protein concentration is increased. These phenomena are driven by the attachment kinetic condition, i.e. the existing deposit grows if protein concentration is larger than S and the mass exchange is proportional to the local value of the orientation-dependent kinetic coefficient; in the opposite situation, i.e. in case protein concentration becomes smaller than S, deposit begins to re-dissolve.

In the case of tissues (of course) the construct cannot re-dissolve since the second member of Eq. (10) cannot be negative. Note that in this case, according to eq. (6b) the growth phenomena are mainly driven by the shear stress distribution i.e. the existing deposit grows if nutrient concentration is not zero and the tissue enlargement (due to "internal cell division" and "production of extracellular matrix") is proportional to the local value of the kinetic coefficient and of the fluid shear stress.

The determination of \( \Delta s \) in eq. (14) requires a well defined ‘interface-reconstruction’ technique (the shape of the construct for a fixed time is not known a priori and must be determined as part of the solution, for the present analysis the non-connecting straight lines technique of Gueyffier et al. (1999) has been used).
3.4 Governing field equations:

In presence of convection in the reactor, the flow is governed by the continuity, Navier-Stokes and species equations, that in non-dimensional conservative form read:

\[ \nabla \cdot \mathbf{V} = 0 \quad (15) \]

\[ \frac{\partial \mathbf{V}}{\partial t} = -\nabla p - \nabla \cdot [\mathbf{V} \mathbf{V}] + S_c \nabla^2 \mathbf{V} + S_c \left( \frac{C}{C_{(o)}} - 1 \right) i_g - S_c \frac{1}{\eta} \mathbf{V} \quad (16) \]

\[ \frac{\partial C}{\partial t} = - \nabla \cdot (\mathbf{V} C) + \nabla^2 C \quad (17) \]

where \( S_c = \nu / D \) is the Schmidt number (\( \nu \) is the kinematic viscosity of the liquid filling the reactor) and \( R_a = \frac{g \beta_{solute} C_{(o)} L^3}{\nu D} \) (the Boussinesque approximation is used to model the buoyancy forces, \( \beta_{solute} \) is the solutal expansion coefficient related to the nutrient in liquid phase). The non-dimensional form of the equations results from scaling the lengths by a reference distance \( L \), the time by \( L^2 / D \), velocity \( \mathbf{V} \) and pressure \( p \) by \( D / L \) and \( \rho_s D^2 / L^2 \) respectively; the initial value of nutrient is \( C_{(o)} \). Note that concentrations are not posed in non-dimensional form ([g cm\(^{-3}\)]).

Assumptions invoked in the development of equations for this continuum model include: laminar flow, Newtonian behaviour of the phases (this implies that the constructs, should be treated as highly viscous fluids), constant phase densities.

Moreover the sample is assumed to be nondeforming and free of internal stress, while the multiphase region (region where nutrients are absorbed and increase of mass occurs) is viewed as a porous material characterized by an isotropic permeability \( \eta \). The term \( -S_c V / \eta \) in equation (16) is the Darcy term added to the momentum equation to damp convection in the solid phase. In the present analysis permeability is assumed to vary according to the Carman-Kozeny equation (Voller and Prakash (1987); Lappa and Savino (2002)) \[ \eta = \left( \frac{1 - \phi}{\phi^3} \right)^3 \phi^2 \]. In practice the effect of \( \eta \) is as follows: in full liquid elements \( 1 / \eta \) is zero and has no influence; in totally solid elements, the final
large value of $1/\eta$ will swamp out all terms in the governing equations and force any velocity predictions effectively to zero.

Since the momentum and species equations are valid throughout the entire domain, explicit consideration need not be given to boundaries between solid, multiphase and liquid regions.

The detailed implementation of the numerical method for the solution of eqs. (14-17) is not described here for the sake of brevity. It proceeds in 4 major stages:

1. Solution of the Navier-Stokes equations according to the MAC method (eqs. 15-16);
2. Solution of the species equation (eq.17);
3. Adjournment of the local values of $C$ at the solid/liquid interface according to eq. (10);
4. Adjournment of the phase field variable distribution (eq. 14).

Parallel supercalculus is used due to the enormous time required for the computations (although the model is two-dimensional). The problem is split in two problems, one parabolic and the other elliptic. A parallel algorithm, explicit in time, is utilized to solve the parabolic equations (momentum and species equations). A parallel multisplitting kernel is introduced for the solution of the pseudo-pressure elliptic equation, representing the most time-consuming part of the algorithm.

A grid-partition strategy is used in the parallel implementations of both the parabolic equations and the multisplitting elliptic kernel. A Message Passing Interface (MPI) is coded for interprocessor communications. For further details see e.g. Lappa (1997) and Lappa and Savino (1999).

4. Results

Numerical simulations carried out using the mathematical models and numerical techniques described in section 3, have provided important and interesting results giving insights into the mechanisms of the growth process for both cases of protein crystals and biological tissues. These numerical results in turn have allowed to identify important analogies (and differences) in addition to those already pointed out in the section devoted to the mathematical models.
Two reference cases have been considered for numerical simulations. These cases correspond to "typical" situations and/or to very important experimental results available in literature (e.g. the excellent experimental results obtained by Freed et al. (1997) dealing with the growth of cartilage tissue). For the sake of simplicity the kinetic coefficient is supposed to be the same for the different sides of the specimen.

As reference case for macromolecular crystal growth (see Figs. 1 and 2) a seed macro-crystal of lysozyme under micro-gravity conditions (g=10^{-4} g_o) is simulated. Hen egg white lysozyme is used as model protein, since it is a well-characterised molecule and it is often used in literature for systematic, controlled in vitro studies of the growth process. The precipitant agent is NaCl. The crystal (1 [mm] x 1 [mm] initial size) is supposed to be fixed (e.g. by glue) to the mean point of the left wall of the reactor (10 [mm] high and 30 [mm] wide test cell) used for the growth process. Growth is obtained from a super-saturated solution with \( C_{\text{lyso}(o)}=5\cdot10^{-2} \) [g cm^{-3}] and \( C_{\text{NaCl}(o)}=2.5\cdot10^{-2} \) [g cm^{-3}] \((\sigma_{(o)}\cong5\), see Otálora and García-Ruiz (1997) for the relationship between salt concentration and lysozyme solubility). The frontier of the domain is supposed to be impermeable to the protein. A grid 300x100 is used to ensure good resolution and grid-independence (see Table I for further details).

As "typical" case for the growth of biological tissues the experiments of Obradovic, Meldon, Freed, Vunjak-Novakovic (2000) and Freed et al. (1997) are considered. According to the geometry of the reactor used by the investigators, a 20 [mm] high and 20 [mm] wide computational domain is simulated (Figs. 3 and 4). According to a grid refinement study (not shown for the sake of brevity) a mesh with 200 points in axial direction and 100 points in radial direction is used. Growth is obtained from a solution with initial concentration \( C_{\text{glucose}(o)}=4.5\cdot10^{-3} \) [g cm^{-3}] (see table II for further details). The frontier of the domain is supposed to be at constant concentration during the growth process (i.e. \( C_{\text{glucose}}=C_{\text{glucose}(o)} \)). The specimen (a disc-shaped cartilage construct having initial size 5 [mm] x 2 [mm]) is supposed to be maintained settling at an approximately steady position within the vessel under normal gravity conditions (dynamic equilibrium of the operative
forces gravity, buoyancy and drag while maintaining a state of continuous free fall). From a mathematical point of view and according to Galileo's invariance principle, this situation corresponds to the specimen at rest as seen in a laboratory frame and the fluid moving upward in the direction opposite to the gravity force with velocity equal to its "terminal velocity" (U). The tissue main components are GAG (glycosaminoglycan) and Collagen (see Table II). The velocity field is supposed to be uniform and parallel at a sufficient distance (about 1 [cm]) from the construct. Its intensity there is assumed to be equal to U (U = 4.64 [cm s\(^{-1}\)]). Its body is supposed to maintain axisymmetric shape during the growth process.

The numerical simulations of these cases elucidate the intrinsic mechanisms of the phenomena and at the same time allow to draw important information and data about similarities and common features.

4.1 Mass transport phenomena occurring in the reactors

Crystals of biological macromolecules are obtained by precipitation from super-saturated solutions and for this reason crystallization can be influenced by gravity. Concentration (density) gradients exist in the crystallizing solutions (as an intrinsic consequence of the crystal-growth process). The growth process, in fact, depletes of protein the liquid surrounding the growing crystal; the related concentration gradient implies a density gradient that, in presence of gravity, induces buoyancy driven convective flow.

These phenomena occur also during experiments in space. In fact, the residual gravity estimated on board the space-shuttle and the International Space Station (ISS) is not zero, but typically of the order of \(10^{-5}\) of earth gravity (see e.g. Otálora, Novella, Gavira, Thomas, García-Ruiz (2001)).

This flow field modifies the protein distribution around the crystal leading to a non-symmetrical concentration pattern (Figs. 1). One recognizes the well-known convective flow pattern for solution growth with a rising ‘plume’ above the crystal. When growth sets in, the solute concentration...
around the crystal seed decreases. With this depletion of the heavier solute, the solution around the crystal becomes lighter and, thus, rises.

In the case of the growth of tissue in the rotating vessel, fluid motion is not due to pluming phenomena but follows from a secondary flow pattern induced by particle sedimentation through the fluid media (on ground conditions) or by laminar flows established when differential rotation rates are chosen for the inner and outer cylinders (microgravity conditions).

The bioreactor was developed by NASA to simulate the weightless environment of space on Earth by putting biological constructs in a growth medium that constantly rotates and keeps them in endless freefall. Of course, the rotating vessel does not really cancel gravity, but ideally maintains continual suspension conditions similar to that experienced by astronauts in the microgravity of space.

The rotating-vessel approach usually involves seeding cells onto 3D polymer scaffolds. The cell/scaffold constructs are placed in the rotating bioreactor that supplies the cells with nutrients and gases and removes wastes.

Many excellent analyses have appeared where the dynamics and the motion of scaffolds and particles of different types and sizes were investigated: see e.g. Wolf and Schwartz (1991, 1992), Gao, Ayyaswamy and Ducheyne (1997a), Meaney, Johnston, Litt, and Pollack (1998), Pollack, Meaney, Levine, Litt and Johnston (2000), Hammond and Hammond (2001), Coimbra and Kobayashi (2002), etc. These studies provided interesting information about the "orbital paths" and trajectories described by particles moving in the rotating reactor as well as about their stable or unstable "equilibrium points" (particular positions that behave as "attractors" for the solid particles). They also tried to estimate the order of magnitude of the stress level acting on the moving particles and some very interesting analytical relationships were elaborated. More comprehensive investigations of the overall mass transfer and fluid dynamic and shear environments in rotating bioreactors were carried out by Kleis, Schreck and Nerem (1990), Gao, Ayyaswamy and Ducheyne (1997b), Begley and Kleis (2000).
In the light of the above studies, it is well known that a particle in suspension culture in the rotating-wall vessel may accelerate through the fluid until it reaches a terminal velocity at which the pull of gravity is balanced by equal and opposite hydrodynamic forces including shear, centrifugal, and Coriolis forces.

Usually, however, these particles do not maintain an approximately steady position within the vessel. Rather they follow almost circular trajectories. In the case of small particles tangential Coriolis induced motion is significant and leads to spiraling of them in the fluid stream with respect to a laboratory reference frame. Due to this effect the particles may fall on large-radius orbits, which periodically strike the vessel wall.

Nevertheless, in the experiments of Freed et al. (1997) and Obradovic et al. (2000), the rotation rate was adjusted so as to suspend each tissue construct about a fixed position within the vessel as viewed by an external observer. In these conditions, a tissue construct is fed as it effectively sieves through the nutrient media. On Earth moreover, in the case of cylindrical scaffolds (Freed et al. (1997)) the settling process tends to align their flat circular areas perpendicular to the direction of motion (see Figures 4). This leads to the formation of a "wake" beyond the floating construct (Figs. 4a-c) and to a depletion zone (Fig. 4d) for the nutrient distribution whose configuration is very similar to that occurring in the case of protein crystal growth due to gravitational solutal pluming phenomena. Due to the fluid skirting around the tissue sample, in fact, a plume-like depletion zone is created above (behind) the tissue.

It is worthwhile to stress how in the (aforementioned) analyses available in literature, the "morphological" evolution of the particle moving in the bioreactor was not taken into account. The "macroscopic" motion of the particle was studied but how this motion may affect the shape-evolution of the growing specimen and how in turn the changing shape may affect the flow around the construct was not investigated (on the contrary these aspects are the main goal of the present analysis, see the next paragraph).
4.2 Morphological instabilities

For both cases of macromolecular crystals in protein reactors and biological tissues in rotating vessels, the growth process is associated to the onset of morphological instabilities (i.e. habit/shape change of the growing constructs). The understanding of these phenomena is crucial in determining generalised criteria to obtain well-controlled growth (i.e. final samples of desired shape and size).

In the case of crystal growth (Figs. 1), the simulations show that from a ‘local’ point of view corners and edges of the crystal are more readily supplied with solute than the centre of faces (this leads to a macroscopic depression around the centre of the faces and therefore to face-morphological-instability, see figs. 2). This is due to the pattern of the protein concentration field around the crystal. As previously discussed, incorporation of the solute into the crystal causes a local depletion in concentration and a solutal concentration gradient to form between the bulk solution and the growth interface. According to eq. (10), the ‘steepness’ of the gradient determines the rate of solute transport to the growth interface, the steepness being (of course) maximum around the corners. Superimposed on this is the fact that a protuberance on the interface sees a higher supersaturation and grows faster than a depression, which sees a lower supersaturation. Note that the onset of morphological instabilities and the existence of ‘depressions’ around the centre of the faces of growing crystals (shown by the present numerical results) is in good agreement with experimental results (Monaco and Rosenberger, (1993)).

Further to the effect associated to the corners more readily supplied with solute, there is an effect due to the convective pattern around the crystal. The ‘face’ (average) growth rate in fact exhibits a different value according to the orientation of the face with respect to the direction of the residual g. Figs. 1 and 2 show that the increase of volume of the crystal is more pronounced for the bottom side than for the upper side i.e. the convection effect results in higher local growth rates near the surface where the flow is incoming and lower local growth rates near the surface where the flow is outgoing. This behaviour can be explained according to two different aspects.
Due to the convective structure of the buoyancy-induced flow pattern, in fact, liquid is transported towards the bottom side and in opposite direction around the upper side. According to this behaviour, liquid regions where the amount of protein available in liquid phase is still large are transported towards the bottom side of the crystal. This increases the growth rate of the lower face (a large amount of protein in solute phase is available for crystal growth). On the other hand, due to the outgoing flow, the depletion zone close to the upper face is distorted and elongated towards the top of the test cell (‘plume’). This leads to a decrease of the concentration gradient (the depletion layer becomes thinner with respect to the bottom side of the crystal) and hence to a decrease of the mass exchange flux between solid and liquid phase. This explains the occurrence of a higher value of the growth rate on the bottom face.

This clearly demonstrates that orientation-dependent growth may occur even if the dependence of the kinetic coefficients on the orientation of the faces is neglected. In this case, it is due to the non-symmetrical concentration pattern around the crystal distorted by the effect of convection according to the direction of the residual g. The growth rate is strongly affected by the mass transport in liquid phase and g-orientation-dependent growth occurs.

The growth process of the cartilage tissue specimen is shown in Figs. 3-4. Each figure of the sequence corresponds to a different snapshot of the growth process.

A toroidal vortex roll is created behind the growing specimen. This behaviour is due to the obstruction created in the fluid flow by the presence of the disc-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 the body in the downstream direction. The two vortices are embedded in a low velocity region (the "wake").

Note that the interaction of the flow entering into the computational domain from the lower boundary with the axisymmetric body embedded in the inner space of the reactor 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 since it enters the computational
domain directed along x and then it is forced to skirt 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 to the presence of the specimen.

This structure is crucial in determining the distribution of surface shear stress and thus the growth behaviour and re-modelling.

This behaviour is evident in Figs. 3 where the growth habit simulation and the progression of cartilaginous matrix deposition are shown in detail. Note that also for this case the predicted (by numerical simulation) onset of morphological instabilities and the existence of ‘depressions’ around the centre of the faces of growing tissue is in very good agreement with the experimental results (Freed et al. (1997) and Obradovic et al. (2000)). Figs. 3, in particular, allow the Reader to compare the numerical results with the experimental ones (Obradovic et al., 2000) by highlighting some characteristic points along the border of the construct (please consider the correspondence between the points A-F in Fig. 3a and Fig. 3b).

A detailed description of these phenomena requires a separation of the analysis for the different faces of the construct as for the case of protein crystal growth.

The ‘face’ (average) growth rate exhibits a different value according to the orientation of the face with respect to the main direction of the flow i.e. an important parameter is the relative direction of the different faces, which induces varying conditions for each face.

Fig. 5 shows in detail how the convection effect results in higher local shear stresses near the surface where the flow is incoming (lower face) and in a lower shear stresses near the surface where the flow is outgoing (upper face). This behaviour 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 upper side (the tissue surface facing the wake is almost plane and
without irregularities). On the contrary, large protuberances are formed on the bottom side due to the strong shear stresses occurring there close to the corners. It is worthwhile to stress how this behaviour is very similar to that described in the case of macromolecular crystal growth.

4.3 Effects related to the increasing size of the samples

Figures 1 show that the convective cell induced by solutal buoyancy forces does not extend throughout the bulk of the protein chamber and is confined to the zone between the right face of the growing crystal and the right wall of the protein reactor. As time passes, the vortex roll shrinks while migrating towards the right wall of the protein chamber. The contraction of the cell and its confinement to a zone close to the right wall are strictly related to the behaviour of the crystal that puffs out due to growth.

There is however a further and more important aspect related to the growing size of the specimen. One must keep in mind that there is an effect related to the fact that the magnitude of the flow is directly proportional to the size of the face upon which it is being generated. Thus, as crystal grow, solutal gradient driven flows can become a major factor for the growth rates. Around the corners in fact the convective flows become very strong and tend to increase the steepness of the concentration gradient there. According to these behaviours, Fig.2 shows that the ‘depth’ of the face depressions (and therefore the difference in gradient steepness between face corner and face centre) is proportional to the size of the crystals.

A similar (from a macroscopic point of view) behaviour occurs in the case of biological tissue growth. The ‘depth’ of the face depressions is proportional to the size of the specimen i.e. it increases during the growth (Figs.3). In this case however, according to eq. (4), the behaviour is not related to the steepness of the concentration gradient of nutrients in solution but depends on the direct effect of the shear stress on the "surface kinetics".

As time passes and the tissue widens, in fact the disturbance in the flow field produced by the presence of the construct becomes larger. The toroidal vortex close to the upper side widens. The expansion of the wake is strictly related to the behaviour of the tissue that puff out due to growth.
At the same time, for the same reason (increasing size of the sample), the shear stresses (and associated growth rates) responsible for the growth of protuberances at the corners of the lower surface increase. This is due to the acceleration of the flow around the corners induced by the progressive reduction of the available cross-sectional area in direction perpendicular to the flow and provides a theoretical explanation for the trend shown in Fig. 6.

5. Discussion

Note that, despite the macroscopic analogies pointed out in section 4, however the cause-and-effect relationships exhibit interesting differences:

- in the case of crystals growing while attached to the walls of the reactor, convective flow is originated by the buoyancy forces ("natural" convection) associated to solutal effects (liquid with impoverished solute that rises being lighter than the surrounding);

- in the case of tissue growing in a rotating reactor about a fixed position within the vessel as viewed by an external observer (due to equilibrium of external forces gravity and drag), convective flow is due to a "forced" convection;

- in the case of organic macromolecular crystal growth, morphology instability follows from the non-uniform distribution of the nutrients around the crystal due to convective effects. Mass transfer is higher close to the corners of the crystals for two reasons: a) a protuberance on the interface sees a higher supersaturation and grows faster than a depression, which sees a lower supersaturation b) convection increases the steepness of the concentration gradient close to protuberances of the surface. There is not any "direct" effect of the convective field on the intrinsic mechanism of growth. The crystals grow according to the local level of supersaturation and according to the steepness of the concentration gradient. Both these effects in turn change according to the intensity of the velocity field;

- biological tissues do not behave as macromolecular crystals; the diffusion of momentum towards the tissue interface alters the manner with which nutrients are incorporated into the tissue mass and the tissue grows; there is a "direct" effect of the fluid-dynamic shear stress on
the "surface incorporations and conversion kinetics" and/or on the "internal cell division" and "addition of extracellular matrix" mechanisms responsible for tissue enlargement (i.e. the physical forces induce changes in cell metabolism and function);

- in the case of crystal growth, the increasing size of the sample plays a role through its effect on the buoyancy plume. The intensity of these plumes in fact depends on the size of the face upon which they are generated;

- in the case of biological tissues, the increase of size of the construct leads to a progressive reduction of the available cross-sectional area in direction perpendicular to the flow. This effect accelerates the fluid laterally skirting around the construct and increases the shear stresses at the lower corners.

6. Conclusions

The present manuscript forms a preliminary but quite exhaustive effort to shed some light on biological tissue growth phenomena from an organic crystal growers point of view. Moreover novel mathematical models have been provided for both topics. They deal with the surface kinetics of the growth process and have been discussed in the same frame of "moving boundary methods" and mathematical models for growing specimens. Numerical simulations of these aspects carried out by using the proposed sophisticated techniques have given insights into the mechanisms of the phenomena allowing at the same time to draw important information and data about similarities and common features. Analogies about the macroscopic behaviour of growing macromolecular crystals and growing biological tissues as well as differences about cause-and-effect relationships have been pointed out. The understanding of these phenomena is crucial in determining generalised criteria to obtain controlled growth (i.e. final samples of desired shape and size).
7. References


Table I: Properties and operating conditions: lysozyme crystal growth

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<td>$v$ [cm$^2$ s$^{-1}$]</td>
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<td>$\rho_c$ [g cm$^{-3}$]</td>
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Table II: Properties and operating conditions: cartilage tissue growth

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<td>$\rho_{\text{GAG}}$ [g cm$^{-3}$]</td>
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<td>$\rho_{\text{collagen}}$ [g cm$^{-3}$]</td>
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</tr>
<tr>
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</tr>
<tr>
<td>a [-]</td>
<td>$\approx 3 \times 10^{-9}$</td>
</tr>
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Figs. 1: Snapshots of growing lysozyme crystal, concentration distribution and velocity field ($g=10^{-4}$ go): (a) $t=3.5 \cdot 10^4$ [s], (b) $t=10.5 \cdot 10^4$ [s], (c) $t=2.8 \cdot 10^5$ [s], (d) $t=4.9 \cdot 10^5$ [s], (e) $t=1.26 \cdot 10^6$ [s], (f) $t=2.42 \cdot 10^6$ [s].
Fig. 2: Growth habit simulation of a seed of lysozyme \( (C_{\text{lys(o)}}=5 \cdot 10^{-2} \, [\text{g cm}^{-3}], \, C_{\text{NaCl(o)}}=2.5 \cdot 10^{-2}) \): snapshots of the crystal shape versus time \( (\Delta t= 3.5 \cdot 10^4 \, [\text{s}]) \).

Fig. 3: (a) Tissue growth habit simulation and progression of cartilaginous matrix deposition: snapshots of the tissue shape versus time \( (\Delta t= 5.2 \cdot 10^4 \, [\text{s}]) \), (b) experimental histological cross-section of cartilage construct cultured for six weeks (Obradovic et al., 2000. Reproduced with permission of the American Institute of Chemical Engineers. Copyright © 2000 AIChe. All rights reserved).
Figs. 4: Snapshots of growing tissue and surrounding velocity field: (a) t= 10 [days], (b) t=22 [days], (c) t=40 [days]; d) Glucose concentration distribution (t=40 [days])
Fig. 5: Nondimensional shear stress distribution (x $10^8$, t=40 [days])}

Fig. 6: maximum shear stress versus t