Ca-alginate hydrogel rheological changes caused by yeast cell growth dynamics

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Interactions between immobilized cell populations and polymer matrixes in the form of hydrogel within micro-beads have complex restrictive action on cell growth dynamics. The forces generated by cell growth and interactions between solvent, network parts and cells influenced this process. It induces structural changes of hydrogel which has the feedback action on cell growth. Rheological response of hydrogel includes both the reversible deformation of domains, as well as the domains partially disintegration which cause permanent irreversible deformation. Estimation of the relationship between cell dynamic environment and cell function offers the possibility for the optimization of various biotechnological processes. The growth of yeast cells within Ca-alginate hydrogel matrix is used as model system for considering such complex phenomena. Structural changes of Ca-alginate hydrogel is modelled using modified general Zener constitutive equation with fractional derivatives. It is suitable for incorporating irreversible effects on macroscopic level. However, the additional consideration of the dynamics of structural changes of Ca-alginate network on mesoscopic level offers the deeper insight into the irreversible nature of deformation caused by cellular local mechanical action. The particular form of free energy functional on mesoscopic level describes various kinds of interactions, which affected the dynamics of cell growth and cause pseudo-phase transition of hydrogel.

Keywords yeast growth; disintegration of Ca-alginate hydrogel

1. Introduction

The entrapment of cells within hydrogel matrixes is a cell immobilization technique attractive for a variety of applications including food technology, pharmacy and biomedicine [1-16]. Of the basic interest for various applications is the choice of material and procedure of micro-bead matrix preparing. Owing to the very gentle, simple and rapid procedure, the entrapment of cells in alginate hydrogels is still the most frequently used method for immobilization [1-17]. Significant attempts have been made to optimize the performances of such beads by ensuring appropriate conditions for their biotechnological functions [3-10]. These functions depend on efficient supply of cells by nutrients, optimal microenvironment conditions for cells inside the bead, decrease of cell leakage from beads, etc. However, much less explanation has been made of the microenvironmental restrictive effects of cell growth. A number of authors have reported early suppress of cell growth for different systems of immobilized cells [1-16]. Some authors also detected such charges, but it is rather hard to describe more in detail the mechanism of that multi component and multilevel process. Therefore, if can be elucidated the relationship between the cell dynamic environment and cell function it is highly possible to find a method by which cell functions can be regulated.

For this purpose, it is necessary to consider the rheological behavior of hydrogel matrix. Significant attempts have been made to examine the rheological response of hydrogel matrix to stresses generated by compression, shear and tension [15-16]. However, little is known about the rheological responses of variously structured matrixes caused by cells growth. The growing cells press to the surrounding and create a new space for further cells growth inside the matrix. Such cell actions are obtained on two time scales, i.e. one is migration time (short-time scale) while the other is growing time (long-time scale). These cell actions result in the matrix structural changes and provoke the complex rheological response. The response includes both the reversible deformation of domains, as well as the domains partially disintegration which cause permanent irreversible deformation. This complex process is influenced by various multi scale interactions, i.e. the interactions between domains themselves as well as the interactions between chains within the domains. Pragmatic approach to such multidisciplinary field sometimes overshadows some serious problems which arise from complexity of multi scale interactions, so adequate interpretation is a major challenge. We consider such complex phenomenon by estimating the growth of yeast cells entrapped within Ca-alginate hydrogel matrix on mesoscopic and macroscopic levels.

Simpson et al. [17] reported that expansion of immobilized cell population induces irreversible structural changes of Ca-alginate network. They consider the growth of βTC3 cell within Ca-alginate matrix with and without washing with $CaCl_2$. Washing treatment causes reconnection of disintegrated parts of network. Microenvironmental growth restriction is more significant for the washing case. However, Pajic-Lijakovic et al. [12-13] obtained significant suppression of yeast cell growth within Ca-alginate matrix in experiments without washing with $CaCl_2$. Irreversible structural change of Ca-alginate network is dominant in such case. Cells are able to partially disintegrate the network and find the new space for growth. However, they fill only 20 % of Ca-alginate micro-bead volume. Irreversible changes of Ca-alginate hydrogel intensify the electrostatic interactions between the free parts of polyelectrolyte chains such as alginate and negatively charged cell membranes. On the other hand, the attractive forces of the network segments tend to keep the

structural integrity and cause the damping of energy dissipation. It is rather hard to interpret more in detail this multilevel process, from the molecular level of polymer network till the cell united actions in clusters. But, its energetic effects can be considered the process modeled in terms of modern thermodynamics and rheology [12-13].

Pajic-Lijakovic et al. [13] considered the mechanism of alginate matrix rheologcal changes by using fractional derivatives on macroscopic level. It enables us to determine system behavior by calculating the order of fractional derivative. Such quantity represents the crude indicator of irreversibility. Formulation of further step in modeling should consider the mechanism of pseudo-phase transition of hydrogel matrix from connected to disintegrate state on mesoscopic level. The pseudo-phase transition represents the consequence of yeast cells local mechanical actions during growth. Pajic-Lijakovic et al. [12] applied "coarse graining" of such multilevel systems to obtained *netto* changes of their free energy. The free energy changes can be represented by Landau-Ginzburg-Wilson (LGW) functional [18-22]. The dynamics of yeast-Ca alginate hydrogel system is described by modeling of the temporal evolution of effective free energy functional towards the equilibrium state.

2. Mechanical properties of Ca-alginate hydrogel and (*Saccharomyces uvarum*) brewer's yeast

Alginate is collective term for a family of polysaccharides produced by brown algae and bacteria. Chemically they are linear copolymers of 1→4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) arranged in a block wise pattern along the chain with homo-polymeric regions of M (M blocks) and G (G blocks) residues interspersed with regions of alternating structure (MG blocks). Polygluronate units in the alginate molecules form a chelated structure with calcium ions called an 'egg box' junction with interstices in which the cations may pack and be coordinated [23]. The junctions between the chains formed in this way are kinetically stable toward dissociation. Polymannuronate units show the normal polyelectrolyte characteristics of cation binding.

Mechanical properties of hydrogel matrix are usually characterized by compression, shear and tension [15-16]. The properties of Ca-alginate hydrogel quantified through the values of Young's modulus, compression modulus and shear modulus are highly dependent on the choice of the alginate polymer and how it was processed. High guluronic acid containing alginate polymers yielded stronger, more ductile hydrogels than high mannuronic acid containing alginates. The properties of Ca-alginate hydrogel also depend on the cross-linker, the gelling environment and storage environment. The Young's modulus ranges from 1 to 10 kPa, the compression modulus ranges from less than 1 kPa to greater than 1000 kPa, while the shear modulus has values in the range of 0.02 to 40 kPa [15-16]. Ca-alginate network represents the dense structure with the effective pore size between 11 to 20 nm [25], which is much less than the averaged diameter of yeast cell (about 6 μm).

The primary function of alginate matrixes in biotechnology applications is to provide mechanical integrity of immobilized cell population through keeping the optimal packing state. Matrixes simultaneously transmitted mechanical signals to the cells and developing population. As Ca^{2+} ions in bucked egg-box junction are being released as the results of action of cells, electrostatic repulsion between carboxylate anions enhances the additional swelling effects of alginate gel. This electrostatic repulsion forces have the feedback action on cell growth [12-13].

A common method to characterize the mechanical properties of cells is by the compression experiments using micromanipulation [24]. In such experiments the cell is compressed between flat parallel plates. Force is measured during the cell deformation up to burst. Single yeast cell behave as hyper-elastic material. The Young's modulus of single cell is equal to 112±6 MPa for cell in exponential phase and 107±6 MPa for cell in stationary phase [26]. Bursting force is in the range 50-200 μN while the corresponding breaking strain is in the range 60-110 %. Yeast cells are stronger than mammalian cells. However, immobilized mammalian cells can also destroy the Ca-alginate network [3-6].

3. Ca-alginate structural changes on macroscopic and mesoscopic levels

3.1 Phenomenological description of Ca-alginate microbead with immobilized yeast cells

We focus on growth of yeast cell population within Ca-alginate hydrogel matrix. The considered system, i.e. micro-bead consists of two subsystems: polymer hydrogel matrix and dispersed cell population. The cell population is entrapped within matrix in the form of separate clusters. The motion of cells is restricted to the motion of cells within the clusters. The averaged initial number of cells per cluster is 1-3, while the final number is about 3000 cells per cluster based on our experimental observations. The interactions between clusters can be neglected based on our experimental observations [12]. The number of clusters is approximately the same during the cell growth. In the absence of external forces, composite system of cells and hydrogel could be treated as isotopic (and homogeneous on scale much larger than the cell size). We point to the influence of mechanical and electrostatical loading arise through

the interactions between cell and matrix subsystems during the cell growth on structural changes of Ca-alginate hydrogel and feedback restrictive action on cell growth.

Both subsystems change their packing states during the relaxation processes obtained at two time scales. The short-time τ represents the migration time of cells, while the long-time t is growing time [27-28]. The number of cells per micro-bead is constant during the short-time period. The long-time t represents the time in which the number of cells per micro-bead changes are present. The complex phenomenon studied can be described through the many relaxation cycles for both, cell population and polymer matrix during the short-time period $\tau \in \left[0,\ \tau_{eq}\right]$ for every numer of cell per micro-bead $N(t)$ obtained at long-time t. For every current equilibrium states at $\left(\tau \to \tau_{eq},\ t\right)$ the external stress generated within the matrix is equilibrated with the internal mechanical stress within the cell population. Further increase of number of cells represents the energetic perturbation for both subsystems.

Considering kinetics of yeast growth in the long-time period, we observed an interesting phenomenon, as can be seen from Fig. 1.

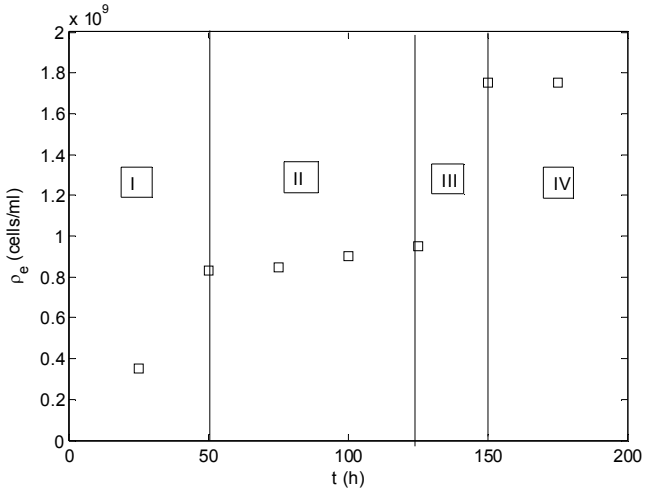

Fig. 1 The increase of the cell number density per volume of micro-bead during the long-time period.

It is presented the number of cells in a bead per unit volume, averaged over the system $\rho_e(t)$ vs. long-time t of cultivation, to line out the trends of cell growth change. For first *ca*. 45 hours can be observed steep increase of $\rho_e(t)$ up to 8 x 10^8 cells/ml with the averaged rate 1.9 X 10^7 cells/(ml h) indicating really good conditions for cell growth. Then, the rate of growth significantly decreases up to 1.23 x 10^6 cells/(ml h) *ca.* 16 times and than almost levels off till $t = 125\ h$ with 1 x 10^9 cells/ml. Then, the rate of growth suddenly increases significantly till $t_{eq} = 150\ h$ and $\rho_e(t)$ to 1.75 x 10^9 cells/ml, when finally levels off.

Initially calls have a lot of free space to grow in already swollen micro-bead. However, when the number of cells increases, they feel elastic resistance of the network. It causes the restrictive effects and the decrease of growth rate. The restrictions of the network increase with further cells expansion. The cell clusters have to push polymer chains and ensure the small increase the free volume. It represents the third part of the curve shown in Fig. 1. At some critical number of cells, clusters have enough power to destroy ionic bonds and ensure the significant new free space. That can be explanation for final increase of growth speed in the forth section of the curve, although all other conditions in the experiment are the same. Breaking of network ionic bonds does not only open a new free space for cell growth. It also leaves new charges at so delivered free groups in the hydrogel. The cell membranes are also partially charged what brings new repulsive interactions into that complex system and probably, finally stops the increase of the number of cells.

The basic question is what type of loading: reversible or irreversible dominantly affect cell growth dynamics and suppress cell growth after $t_{eq} = 150\ h$ in spite of the fact that cells fill only 20 % of micro-bead volume. Reversible loading mechanicaly influences cell growth during the generation the elastical deformation of hydrogel. Irreversible loading becomes dominant for the case of partial disintegration of hydrogel. Such irreversible nature of deformation intensified electrostatic repulsion betwen the some charged parts of free alginate chains and cell membranes. External loading of cells caused by generated stress within Ca-alginate hydrogel has the impact on changes the packing state of cells within clusters.

Yeast population naturaly changes the packing state during the period of growth without external compression [29]. The exponential period of growth corresponds to lower density population while the matured population becomes more compact The external loading of immobilized cell population additionaly contribute to the changes of the packing state.

It causes to the decrease of the averaged distances between cells and intensified the interactions between cells within clusters which can restrict cell growth dynamics [30].

3.2 The rheological model of structural changes of Ca-alginate hydrogel on macroscopic level

The rheological model is developed to elucidate the mechanism of Ca –alginate matrix deformation caused by cells action. Both subsystems, hydrogel and cell population relax during the short-time period $\tau \in \left[0,\ \tau_{eq}\right]$ for every number of cells $N(t)$ obtained at long-time t.

The rheological response of hydrogel includes both the reversible deformation of domains, as well as the domains partially disintegration which cause permanent irreversible deformation. The change of effective volume of hydrogel represents the quantitative indicator of matrix rheological response to cells action. The effective volume for hydrogel subsystem during the growing-time period is expressed for time sets $\left(\tau_{eq},\ t\right)$ as:

$$V_{eff}\left(\tau_{eq},t\right)=V_b\left(\tau_{eq},t\right)\left(1-\phi\left(\tau_{eq},t\right)\right) \tag{1}$$

where $V_b\left(\tau_{eq},t\right)$ is micro-bead volume, $\phi\left(\tau_{eq},t\right)$ is volume fraction of cells inside the micro-bead. Micro-bead volume increases with time, which is experimentally obtained [12]. At the same time, the term $1-\phi\left(\tau_{eq},t\right)$ decreases with time due to cell growth. In general, two rheologial trends can be observed. The first is tinning behavior due to reversible, elastically deformed hydrogel. This behavior causes the decrease of the effective volume of hydrogel subsystem. The second trend is additional swelling due to partially disintegration of hydrogel, causes the increase of the effective volume of hydrogel. Based on our experimental observation calculated effective volume of hydrogel changes from $2.67\text{x}10^{-4}$ to $1.13\text{x}10^{-3}$ ml [13]. Such increase of the effective volume indicates irreversible deformation of hydrogel as dominant. It represents the result of partial disintegration of hydrogel which causes electrostatic repulsion between relatively stiff chains of polyelectrolytes as alginate. This part of the process causes the swelling effects.

The stress generated within hydrogel matrix represents the sum of the reversible and irreversible contributions. It is expressed as:

$$\tilde{\sigma}_{m\,T}\left(\tau_{eq},t\right)=\tilde{\sigma}_{m\,R}\left(\tau_{eq},t\right)+\tilde{\sigma}_{m\,I}\left(\tau_{eq},t\right) \tag{2}$$

where $\tilde{\sigma}_{m\,T}\left(\tau_{eq},t\right)$ is the total stress generated within matrix, $\tilde{\sigma}_{m\,R}\left(\tau_{eq},t\right)$ and $\tilde{\sigma}_{m\,I}\left(\tau_{eq},t\right)$ are reversible and irreversible parts of the stress, respectively. Reversible part of stress is purely mechanical stress while the nature of the irreversible part of stress is the combination of the mechanical and electrostatic. Reversible part of stress decreases while irreversible part of stress increases as the result of cells action during period of growth. It results in the increase of the total stress generated within matrix. After the relaxation cycle at $\tau=\tau_{eq}$ for every number of cells $N(t)$ obtained at long-time t the stress generated within matrix is equilibrated with the stress generated within cell population:

$$\tilde{\sigma}_{m\,T}\left(\tau_{eq},t\right)=\tilde{\sigma}_{c}\left(\tau_{eq},t\right) \tag{3}$$

where $\tilde{\sigma}_{m\,T}\left(\tau_{eq},t\right)$ is the stress tensor within matrix and $\tilde{\sigma}_{c}\left(\tau_{eq},t\right)$ is the stress tensor within cell population. The stress generated within cell population depends on cell packing state and can be expressed as function of cell number density per micro-bead. Pajic-Lijakovic et al. [13] expressed the reversible part of stress generated within matrix as:

$$\sigma_{m\,R}\left(\tau_{eq},t\right)\sim\Delta\rho\left(\tau_{eq},t\right) \tag{4}$$

where $\sigma_{m\,R}\left(\tau_{eq},t\right)$ is the component of the reversible part of stress tensor generated within matrix, $\Delta\rho\left(\tau_{eq},t\right)=\rho\left(\tau_{eq},t_{eq}\right)-\rho\left(\tau_{eq},t\right)$ is the cell number density difference while $\rho\left(\tau_{eq},t_{eq}\right)$ is the equilibrium number density of cells per micro-bead volume. Cell number density per micro-bead is expressed as: $\rho\left(\tau_{eq},t\right)=\dfrac{N(t)}{V_b\left(\tau_{eq},t\right)}$.

Considering structural changes of Ca-alginate hydrogel correspond to viscoelastic behavior. Zener-type constitutive equation is widely used for describing the viscoelastic behavior for various systems. This model equation should be modified for describing the specific effects of energy dissipation due to interactions between cells with hydrogel parts and interactions between hydrogel parts themselves. Consideration of such complex phenomena needs using the constitutive equation with fractional derivatives [31-33]. Pajic-Lijakovic et al. [13] formulate the long-time evolution of reversible part of stress within hydrogel based on modified Zener-type equation as:

$$\sigma_{m\,R}\left(\tau_{eq},t\right)=\eta\ {}_0D_t^{\alpha}\left(\lambda_m\left(\tau_{eq},t\right)\right) \tag{5}$$

where η is the effective viscosity, $\lambda_m\left(\tau_{eq},t\right)$ is the component of effective deformation tensor of hydrogel which includes both reversible and irreversible contributions. The volumetric deformation and generated stress is isotopic on macroscopic level, which is in accordance with the experimentally observed uniform expansion of the micro-bead. Riemann-Liouville's fractional derivative [34-35] in Eq. 5 is given by:

$$ {}_0D_t^{\alpha}\left(\lambda_m\left(\tau_{eq},t\right)\right)=\frac{1}{\Gamma\left(1-\alpha\right)}\frac{d}{dt}\int_0^t\frac{\lambda_m\left(\tau_{eq},t'\right)}{\left(t-t'\right)^{\alpha}}dt' \tag{6}$$

where α is the order of fractional derivative and $\Gamma\left(1-\alpha\right)$ is the gamma function. If parameter α is equal to $\alpha=0$, we obtain ${}_0D_t^{0}\left(\lambda_m\left(\tau_{eq},t\right)\right)=\lambda_m\left(\tau_{eq},t\right)$. The proportionality between the volumetric stress component and the strain implies the elastic Hookean behavior. When $\alpha=1$, the corresponding gamma function $\Gamma\left(1-\alpha\right)\to\infty$ and the fractional derivative is not defined. However, it can be shown, that in the limit when $\alpha\to 1$, follows ${}_0D_t^{\alpha}\left(\lambda_m\left(\tau_{eq},t\right)\right)\to\frac{d\lambda_m\left(\tau_{eq},t\right)}{dt}$, where the dot denotes the first time derivative. In this case, the proportionality between the volumetric stress component and the rate of deformation $\frac{d\lambda_m\left(\tau_{eq},t\right)}{dt}$ implies the dissipative behavior. When parameter α is in the range $0\le\alpha\prec 1$, Eq. 1 describes the specific rheological behavior of dumped dissipative phenomena. The component of effective deformation tensor of hydrogel can be obtained from:

$$\lambda_m\left(\tau_{eq},t\right)=\left(\frac{V_{eff}\left(\tau_{eq},t\right)}{V_{eff\,0}}\right)^{1/3} \tag{7}$$

where $V_{eff\,0}$ is initial effective volume of hydrogel while $V_{eff}\left(\tau_{eq},t\right)$ is effective volume of hydrogel given by Eq. 1.

The value of the order of the fractional derivative α provides deeper understanding of rheological mechanisms producing dissipation effects as described above. For that purpose the following iterative procedure is developed: (1) the volumetric deformation due to cell growth is calculated from Eq. 7; (2) as the first guess for the value of the parameter α is chosen 0.1; (3) the corresponding fractional derivatives for every moment of long-time t are calculated numerically (using Grunwald method [36]); (4) the values for cell number densities per micro-bead are predicted from the model; (5) the cell number densities obtained from the model are compared with cell number densities obtained experimentally.

The best agreement between experimental and calculated values from the model is obtained for $\alpha=0.96$ [13]. The closeness of the optimal value for α to one indicates irreversible deformation due to cell growth as dominant.

3.3 The thermo-dynamical model of structural changes of Ca-alginate hydrogel on mesoscopic level

The order of fractional derivative (Eq. 5) represents the crude indicator of irreversibility on macroscopic level. Formulation of further step in modeling is detail consideration of the irreversible structural changes of hydrogel caused by local mechanical actions of cells. Cell actions depend on cell number density per micro-bead. Macroscopically, it is formulated using Eqs. 3-4. Local cell actions depend on local cell number density per micro-bead domain $\rho\left(x,\tau_{eq},t\right)$. Cell number density represents the sum of local contributions expressed as:

$$\rho\left(\tau_{eq},t\right)=\frac{1}{V_b\left(\tau_{eq},t\right)}\int_0^{V_b\left(\tau_{eq},t\right)}\rho\left(x,\tau_{eq},t\right)\,dv\left(x,\tau_{eq},t\right) \tag{8}$$

where $\rho\left(x,\tau_{eq},t\right)$ is local cell number density per meso-domain, $V_b\left(\tau_{eq},t\right)$ is the volume of nearly spherical micro-bead with the radius $R_b\left(\tau_{eq},t\right)$ which is equal to $V_b\left(\tau_{eq},t\right)=\frac{4}{3}\pi\,R_b^3\left(\tau_{eq},t\right)$, $dv\left(x,\tau_{eq},t\right)$ is the volume increment of micro-bead. The volume increments represent the thin layers equal to $dv\left(x,\tau_{eq},t\right)=r\left(x,\tau_{eq},t\right)^2\pi\,dx$, while $r\left(x,\tau_{eq},t\right)^2=x\left(2R_b\left(\tau_{eq},t\right)-x\right)$. Considering meso-domains represents $10\ \mu m$ thin layers of micro-bead based on experimental observation [12].

The irreversible structural changes of hydrogel could be quantified using number density of disintegrated junction zones per micro-bead volume $\psi\left(\tau_{eq},t\right)$. The effective volume of hydrogel expressed by Eq. 1 can be correlated with

the number density of disintegrated junction zones, i.e. $V_{eff}\left(\tau_{eq},t\right)\sim\psi\left(\tau_{eq},t\right)$. It represents the sum of local contributions as $\psi\left(\tau_{eq},t\right)=\frac{1}{V_b\left(\tau_{eq},t\right)}\int_0^{V_b\left(\tau_{eq},t\right)}\psi\left(x,\tau_{eq},t\right)\,dv\left(x,\tau_{eq},t\right)$ (where $\psi\left(x,\tau_{eq},t\right)$ is the local number density of disintegrated junction zones).

In further modeling consideration, we are interesting in pseudo-phase transition of hydrogel from connected to disintegrated state caused by various kinds of interactions between cells, between cells and hydrogel and between a parts of hydrogel themselves. Thermodynamics approach is suitable for describing such multi-level process through particular form of free energy functional which includes various affinity contributions $F\left[\rho\left(x,\tau_{eq},t\right),\psi\left(x,\tau_{eq},t\right)\right]$. Free energy functional represents the modification of Ginzburg-Landau-Wilson free energy functional [18-22]:

$$F\left[\rho\left(x,\tau_{eq},t\right),\psi\left(x,\tau_{eq},t\right)\right]\approx\int dx^3\;V\left[\rho\left(x,\tau_{eq},t\right),\psi\left(x,\tau_{eq},t\right)\right] \tag{9}$$

where $V\left[\rho\left(x,\tau_{eq},t\right),\psi\left(x,\tau_{eq},t\right)\right]$ is the potential function. Pajic-Lijakovic et al. [12] formulate the potential function based on modified form of equation proposed by Munoz et al. [21] as:

$$V\left[\rho,\psi\right]\approx-\frac{\mu}{2}\rho^2+\frac{\alpha}{2}\;\rho^2\;\psi+\frac{\beta}{3}\rho^3-\frac{\nu}{3}\psi^3 \tag{10}$$

where μ, α, β, ν are supposed to be constants which should be physically described accordingly with the development of the model proposed. The first term has to represent the energy input, generated from growing cells. The second term will represent the energy dissipation part due to interactions between cells and the parts of hydrogel, which cause the disintegration. The third term will represent the energy dissipation part due to interactions between cells itself. The forth term will represent the resistance of hydrogel to disintegration. The changes of free energy functional indicate temporal evolution of the states of both subsystems.

Langevin-type equation is widely used for modeling the phase transition within various systems [19]. Munoz et al. [20-22] expanded and modified such modeling equation for describing temporal evolution of the dynamical systems with absorbing state phase-transitions. Pajic-Lijakovic et al. [12] formulate the system of Langevin-type model equations for considering the pseudo-phase transition of Ca-alginate network caused by cell growth as the modification of so called model C [19].

$$\frac{\partial\rho\left(x,\tau_{eq},t\right)}{\partial t}=-\Gamma\frac{\delta F\left[\rho\left(x,\tau_{eq},t\right),\psi\left(x,\tau_{eq},t\right)\right]}{\delta\rho\left(x,\tau_{eq},t\right)}+\rho\left(x,\tau_{eq},t\right)\;\eta_\rho\left(x,\tau_{eq},t\right) \tag{11}$$

$$\frac{\partial\psi\left(x,\tau_{eq},t\right)}{\partial t}=\Gamma\frac{\delta F\left[\rho\left(x,\tau_{eq},t\right),\psi\left(r,t\right)\right]}{\delta\psi\left(x,\tau_{eq},t\right)}+\psi\left(x,\tau_{eq},t\right)\;\eta_\psi\left(x,\tau_{eq},t\right) \tag{12}$$

where x is the distance of 10 μm-thin layers from center of the bead, Γ determines the rate of long-time relaxation towards the equilibrium state. The second terms of Eqs. 11-12 represent the multiplicative noises while $\eta_\rho\left(x,\tau_{eq},t\right)$ and $\eta_\psi\left(x,\tau_{eq},t\right)$ are Gaussian while noises. The multiplicative noises terms describe external micro-environmental non-homogeneity inside the micro-bead.

The local number density of disintegrated junction zones can be expressed in suitable form for further modeling consideration:

$$\psi\left(x,\tau_{eq},t\right)=\xi\;y\left(x,\tau_{eq},t\right) \tag{13}$$

where ξ is the specific volume of disintegrated junction zone and $y\left(x,\tau_{eq}t\right)$ is the local volume fraction of disintegrated junction zones. Such volume is higher than the volume of connected junction zones as the result of electrostatic repulsive interactions between the charged parts of free alginate chains. It is in accordance with the increase of effective volume of hydrogel (Eq. 1).

The final form of model equations is obtained after introducing Eq. 9 in Eqs. 11 and 12 as:

$$\frac{\partial\rho}{\partial t}=\mu^*\;\rho-\alpha^{**}\;y\;\rho-\beta^*\;\rho^2+\rho\;\eta_\rho \tag{14}$$

$$\frac{\partial y}{\partial t}=\frac{\alpha^*}{2}\rho^2-\nu^*y^2+y\;\eta_y \tag{15}$$

where $\mu^* = \Gamma\ \mu$ is the effective kinetic constant, $\alpha^{**} = \Gamma\ \alpha\ \xi$ represents the magnitude of repulsive interactions between the same charged parts of alginate chains, causing by disintegration of hydrogel structure, $\beta^* = \Gamma\ \beta$, represents the magnitude of interactions between cells themselves, which also restricted the cell growth, $\nu^* = \Gamma \nu\ \xi$ represents the magnitude of attractive interactions between the parts of hydrogel structure, which try to save hydrogel structural connectivity, $\alpha^* = \Gamma\ \alpha$ represents the kinetic constant for disintegrated junction zones formation as the consequence of cell growth. The first, the second and the fourth terms of eq. 14 are incorporated into the effective rate of cell growth which incorporates microenvironmental restrictive effects:

$$r_{\rho_{eff}}\left(x,\tau_{eq},t\right) = \left(\mu^* - \alpha^{**} y\left(x,\tau_{eq},t\right) + \eta_\rho\left(x,\tau_{eq},t\right)\right)\ \rho\left(x,\tau_{eq},t\right) \qquad (16)$$

where the first term is the kinetic term, while the second, coupled term represents the negative feedback restriction to cell growth.

Eqs. 14 and 15 are solved numerically using the method of finite differences starting with the initial conditions: a) at $t = 0$ for $0 \le x \le R_0$ where $R\left(\tau_{eq}, t = 0\right) = R_0$ is the initial micro-bead radius, the initial cell concentration is equal $\rho\left(x,\tau_{eq},\ t = 0\right) = \rho_0$, while the initial local volume fraction of disintegrated junction zones inside the hydrogel subsystem is $y(r,0) = 0$; b) at equilibrium state for cells, experimentally obtained after $t_{eq} = 150\ h$, for $0 \le x \le R_{eq}$ ($R\left(\tau_{eq}, t_{eq}\right) = R_{eq}$ is the equilibrium micro-bead radius), the total volume fraction of disintegrated junction zones should be equal or higher than total volume fraction of cells i.e. $y\left(\tau_{eq}, t_{eq}\right) \ge \phi\left(\tau_{eq}, t_{eq}\right)$. The total volume fraction of disintegrated junction zones represents the sum of the contributions for all thin layers inside the bead. The volume fraction of cells $\phi\left(\tau_{eq}, t_{eq}\right)$ is experimentally determined.

The model prediction of the total volume fraction of disintegrated junction zones after 150 h is approximately $y\left(\tau_{eq}, t_{eq}\right) = 0.24$, while the experimental value of volume fraction of cells is $\phi\left(\tau_{eq}, t_{eq}\right) = 0.192$ [12].

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