Nonlinear Behaviors in Gene Therapy

Theoretical and experimental aspects

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Gene therapy represents a promising method for treating genetic disorders or diseases consisting in the transfer of genetic material (DNA) to cells in order to substitute or to slow down the evolution of the defective gene in cells. Theoretical models to predict DNA release are difficult to build in the classical approach of continuous and differentiable physical quantities, due to the high number of interdependent phenomena that occur simultaneous. The article presents a theoretical model based on the fractal theory of motions in the form of Scale Relativity Theory to describe nonlinear behaviors in gene therapy. Correlations of the theoretical model with experimental data are also observed.

Keywords: gene therapy, nonlinearity, release, transfer of genetic material

The determinism does not necessarily imply either regular behavior (periodic movements, autostructures, etc.) or predictability in the behavior of polymeric systems. In the linear analysis, on which the standard physics of polymers is based almost exclusively, the unlimited predictability was an automatic quality of polymeric system dynamics. The development of nonlinear analysis and the discovery of laws governing chaos has demonstrated not only that the reductive analysis method, to which the polymer physics has been limited so far, has limited applicability, but also that unlimited predictability is not an attribute of polymer systems, representing, in fact, a natural consequence of their simplification by linear approach. There are only few behaviors in which non-linearity and chaos highlight common manifestations (the superstructure, the glass transition, the flammability, etc.), namely an universality in the laws that dictate dynamics in polymeric systems [1-3].

The behavior of the polymers is determined by the conditions of polymers production [4-7] and by the conditions in which they are used [8]. The nonlinearity and chaoticity of any polymeric system are both structural and functional, the interactions between its structural units determining mutual microscopic-macroscopic, local-global, individually-collectively conditionalities. In such a framework, the universality of the dynamic laws of the polymeric systems becomes natural, obvious and have to be reflected in the mathematical procedures used. Some authors are increasingly discussing about holographic implementations in describing the dynamics of polymer systems (fractal paradigm of the nature) [9].

The usual physical models used in describing the dynamics of the polymeric systems are based on the assumption, otherwise still unjustified, of the differentiability of the physical quantities used to describe their evolution [1, 3]. The success of these models have to be understood gradually / sequentially, in areas where differentiability and integrability are still valid. The differentiable and integrable mathematical procedures fails when we intend to describe evolutions, since only nonlinearity and chaoticity involve them.

In order to describe evolutions, however remaining tributary to differentiable and integrable mathematical procedures, it is necessary to explicitly introduce the scale resolution in the expressions of the physical variables that describe evolutions and implicitly in the expressions of the fundamental equations that govern them [10].

This means that any physical variable, dependent in the classic way, both on the spatial and time coordinates, depends, in the new mathematical *structure*, on the scale resolution. In other words, for example, instead of operating with a single physical variable, described by a strictly non-differentiable mathematical function, we will only work with approximations of this mathematical function obtained by mediating it at different scale resolutions. Consequently, any physical variable used to describe dynamics in polymeric systems will function as the limit

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of a family of mathematical functions, non-differentiable for a null scale resolution and differentiable for a nonzero scale resolution [11, 12].

This way of describing the dynamics of polymeric systems, for which measurements are performed at finite-scale resolutions, obviously implies the development of both new geometric structures and physical theories, compliant with these geometric structures, for which the motion laws, invariant to spatial and temporal transformation, are also invariant at scale resolution transformations. In our opinion, such a geometric structure can be one based on the concept of fractal and the corresponding physical model, described in the Theory of Scale Relativity [11, 12].

From such a perspective, holographic implementations in describing the dynamics of polymeric systems are explained by the movements of polymer structural units on continuous, but non-differentiable curves (fractal curves) [2, 3, 11].

Several consequences are obvious: i) constrained movements on continuous and differentiable curves in an Euclidean space are substituted with movements free of any constraints on continuous, but nondifferentiable curves in a fractal space; ii) the motion curves acts both as the geodesics of a fractal space and as the current lines of a fractal fluid; iii) the structural units of any polymeric system are substituted with their own geodesic, any external constraint being interpreted as a selection of geodesics based on local-global/entire-part compatibility, etc.; iv) for time scales larger with respect to the inverse of the highest Lyapunov exponent [13, 14], deterministic trajectories can be replaced by families of potential trajectories and the concept of defined positions by that of probability densities.

In such conjecture, a holographic implementation, describing nonlinear behaviors in gene therapy, using the Scale Relativity Theory [11, 12], are presented. Moreover, correlations of the theoretical model with experimental data in DNA release are, also, analyzed.

Experimental part

In order to treat genetic disorders or diseases [15, 16], gene therapy represents a promising method; it consist in the transfer of genetic material (DNA) to cells in order to substitute or to slow down the evolution of the defective gene in cells. Gene therapy can be done by the so-called *vectors*, virals or non-virals, able to compress the nucleic acid, at least up to the limit of the biological barriers. More and more, the non-viral vectors are preferred against viral ones due to: i) their ability to be laboratory synthesized in a multitude of entities with reproducible structural characteristics and functional behaviors; ii) their convenient use in transfection protocols; iii) they produce a minimized immunologic response.

The experiments aimed to obtain double stranded DNA (dsDNA) carriers with high transfection efficiency and no acute toxicity**.** Among the synthetic polymers envisaged for the design of non-viral vector, sterically exposed polycations proved to be good reversible transporters for dsDNAs **[**17-24**]**. From these, although cytotoxic, polyethyleneimines (PEI) with low-to-medium molecular weight (5 to 25 kDa) was considered an acceptable option for packing and dsDNA transport.

On the other hand, the design of hybrid nanoplatforms was envisaged due to their functions: multivalent dsDNA binding sites, membrane penetration and anti-opsonisation function. For this, C60 fullerene was chosen due to its nanometric core and its promising biomedical applications [17]. In this context, C60 fullerene-polyethyleneimine (C60-PEI) nanoconjugates, in which hyperbranched low molecular weight PEI (2 kDa) organizes around hydrophobic C60, like a dendrimer-like core structure, were synthesized as dsDNA carriers, following the procedure from [17]. Having in view the use of these vectors in the development of a gene-activated scaffold platform, C60-PEI labeling via 5-fluorescein isothiocyanate (C60-PEI-FITC) was performed [24].

Since matrix mediated gene transfer strategy is considered to enhance gene delivery, increase the extent and duration of transgene expression and insure a safe profile for gene therapy, the polyplexes formed between C60-PEI conjugates as gene delivery vector and salmon dsDNA were excapsulated in a 3-D matrix, a bioinspired hybrid cryogel, containing natural/synthetic polymers (atelocollagen, hyaluronic acid derivative, poly(å-caprolactone)), and polyethylenimine functionalized nano-hydroxyapatite (CH10P10/HAp25-15).

For a nitrogen to phosphorus ratio N/P=10, further studies were conducted by examining free dsDNA and vector release from the matrix systems by means of UV-VIS (for C60-PEI/salmon dsDNA) and fluorescent spectro-photometry (for labeled carrier C60-PEI-FITC/salmon dsDNA). The experiments were performed for 72 days (1749 h) in dynamic conditions: on a temperature controlled shaker at 37^{0}C, 200 rpm, similar to physiological conditions.

Results and discussions

As shown in figure 1, an initial burst of dsDNA release is evident for all investigated systems, mostly in the first 2h with an increased effect in the dynamic system. However, even the released naked dsDNA amount with respect to the initial dsDNA (33.6 wt%) is lower than that reported for combined systems comprising dsDNA vector in a 3D matrix, where it was situated between 60 and 80%.

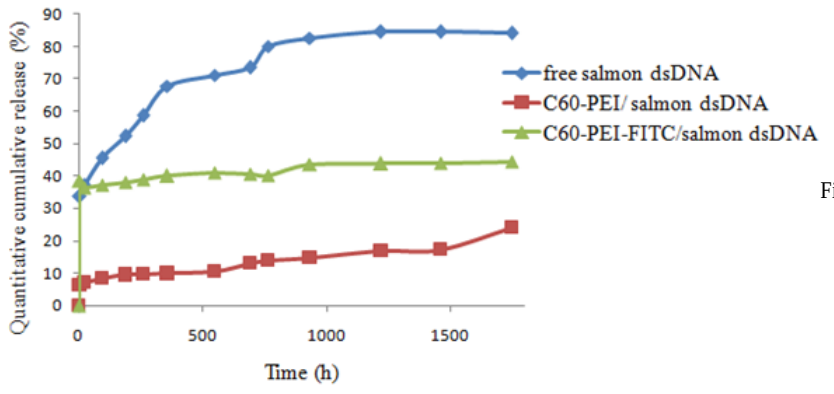

Fig. 1. The release of free salmon dsDNA and salmon dsDNA delivery vector from 3D polymeric matrix

For all kinetic plots, an important slope change is observed in the 0-100 h time interval, and a plateau is reached after about 700 h when the majority of the still existing conjugates appear to remain associated to the scaffold. These aspects are directly related to the matrix degradation behavior.

Theoretical considerations

Taking into account the above mentioned, next we will work in the dynamics of the polymeric systems with probability density P, invariant fractal variable both in relation to the spatial and time coordinates, as well as with the scale resolution. In other words, fractalization through the stochasticity x=x(t) will be described by the probability density P, functional both on x and on t in the form P=P(x,t). The evolution of this probability density satisfies a Fokker-Planck fractal equation which in the unidimesional case is written [5, 6]:

$$\partial_t P(x,t) + \partial_x[V_x P(x,t)] - \lambda (dt)^{(2/D_F)-1} \partial_{xx}[P(x,t)] = 0 \quad (1)$$

where V_x is the velocity of the fractal probability current density, λ is the coefficient associated to the fractal-non-fractal transition, dt is the scale resolution and D_F is the fractal dimension of the trajectories [13, 14]. Equation (1) admits analytical solutions only in very special situations. For example, for $V_x=-\eta x$ with η = cont., the result of the integration for equation (1) is the function [23, 24]:

$$P(x,t) = \frac{1}{\sqrt{2\pi\left(\frac{\lambda}{\eta}\right)(dt)^{(2/D_F)-1}[1-exp(-2\eta t)]}} \; exp\left\{\frac{[x-x_0\,exp(-\eta t)]^2}{2\left(\frac{\lambda}{\eta}\right)(dt)^{(2/D_F)-1}[1-exp(-2\eta t)]}\right\} \quad (2)$$

with x_o=cont. Using the substitutions, adimensional variables:

$$\tau = \eta t,\ \xi = \frac{x}{\sqrt{2\left(\frac{\lambda}{\eta}\right)(dt)^{(2/D_F)-1}}},\ \xi_0 = \frac{x_0}{\sqrt{2\left(\frac{\lambda}{\eta}\right)(dt)^{(2/D_F)-1}}},\ \mathcal{P}(\xi,\tau) = \sqrt{2\pi\left(\frac{\lambda}{\eta}\right)(dt)^{(2/D_F)-1}}\,P(x,t) \quad (3)$$

the relation (2) becomes:

$$\mathcal{P}(\xi,\tau) = [1-\exp(-2\tau)]^{-1/2}\, exp\left\{-\frac{[\xi-\xi_0\,exp(-\tau)]^2}{[1-exp(-2\tau)]}\right\} \quad (4)$$

The fact that P simultaneously operates on two manifolds, one of the spatial coordinates and time and the other of the scale resolutions, allow us to perform various *isometries* such as immersing between these two manifolds, size compactification [2, 14]. In this context, any variable that will describe the dynamics of a polymeric system will be defined as a product between its average value at various scale resolutions and the probability density constructed by adequate isometry. Such formalism has been applied in the study of the physical systems dynamics both at microscale [25-27], mesoscale [28, 29] and macroscale [30, 31].

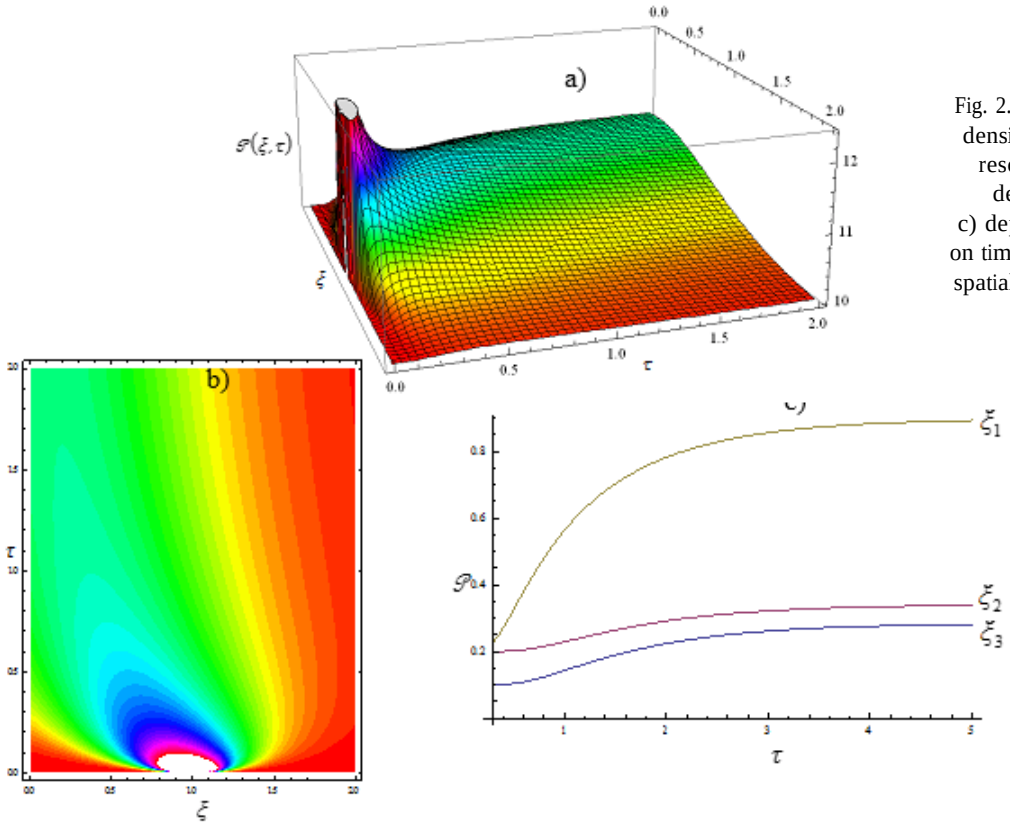

Fig. 2. The dependence of probability density on time and on spatial scale resolution: a) three dimensional dependence; b) contour plot; c) dependence of probability density on time for three distinct values of the spatial scale resolution ξ_1=1.4, ξ_2=1.7, ξ_3=0.6 relative to the the reference value ξ_o=1

For example, we present in figure 2a-c an isometry type obtained by immersing the *spatial coordinates manifold* in that of the *spatial scale resolutions manifold*. From such a perspective, figure 2a corresponds to the 3D dependence of the probability density on time and on the spatial scale resolution, figure 2b contour plot, while in figure 2c the dependence of probability density on time for three distinct values of the spatial scale resolution relative to the its reference value.

The comparison of figures 1 and 2c shows a high degree of similarity between the two, suggesting that the theoretical model can describe, at least qualitatively, the evolution of dsDNA release from the given systems. In such a frame, the product of the average value of dsDNA released at different scale resolutions and the probability density describes the time evolution of the release process.

Let's note that at the initial moment there is a global scale of phenomena and so the average value will be identified with the initial value.

Conclusions

Nonlinear behaviors in gene therapy by means of a holographic implementation using the fractal model of motion in the form of Scale Relativity Theory are presented. So, through a Fokker-Planck fractal equation, various isometries between the *spatio-temporal coordinates manifold* and *scale resolutions manifold* can describe different nonlinear dynamics and, implicitly, nonlinear behaviors in the transfer of genetic material (DNA) to cells, i.e. on gene therapy. In such context, correlations of the theoretical model with experimental data in data release (more precisely, the release of free salmon dsDNA and salmon dsDNA delivery vector from 3D polymeric matrix) are analyzed.

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