MULTI-LAYER OSCILLATORY SPHERICAL NET MODEL OF MOUSE *ZONA PELUCIDA*

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Abstract

Zona Pelucida (ZP) is multi-layer mesh-like 3D structure that surrounds mammalian eggs. One of its biological functions is selectivity regarding sperm penetration. Oscillatory spherical net model of mZP is suitable for explain initial stadium of sperm penetration. This model is single-layer model. On a basis of single-layered oscillatory spherical net model of mZP we proposed improved double layer models. Due to visco-elastic properties of ZP and its importance for mechanism of sperm penetration double layered oscillatory net model of mZP has fractional order properties. Oscillatory behavior of this double-layered system is discussed.

Key words: zona pelucida, multi-layer oscillatory spherical net model, oscillations, coupled meshes

1. Introduction

Zona Pelucida ZP is dynamical 3D structure that changes its structural organization before and after fertilization, which has repercussions on its mechanical properties.
"*During maturation both ZP layers displayed a fine filaments network whose length increased while thickness decreased. After fertilization, filaments partially recovered the immature features, appearing again shorter and thicker"[*1, 2].
On scanning (SEM) and transmission (TEM) electron microscopy two basic ZP layers could be identified: outer with rough spongy appearance on SEM and inner with smaller fenestrations and smooth fibrous network. This structural organization is evident for almost all mammalian ZP-s in mature oocyte ready for fertilization [3,4]. ZP has similar morphology on SEM and TEM in almost all species observed although the molecular composition varies among different species [4].
De facto, ZP is an extracellular matrix (ECM) composed of long, cross linked filaments that build 3D mash –like structure that is created during the period of oocyte growth. In mice, during period of 2-3 weeks, the ZP glycoproteins are synthesized and secreted by growing oocytes and assembled into ECM- Zona pelucida [5].
Although the assembling pattern of ZP is still not known in details it is known which part of ZP molecules are responsible for assembling of ZP glycoproteins and which regulate polymerization

of nascent ZP glycoproteins [5]. Their results showed that ZP domain functions as a polymerization module and suggested a general mechanism for assembly of all ZP domain proteins based on coupling between proteolytic processing and polymerization [5].

Differences in structural organizations among inner and outer ZP layers are probably related to its different mechanical properties [2] and may affect mechanism of sperm penetration trough ZP thickness as well as poyspermy block [1].

Advantage of oscillatory spherical net model of mZP [6, 7] in comparison to biochemical models of fertilization is that it does not deal with single molecules involved in receptor recognition and attachment between oocyte and sperm membranes. The oscillatory spherical net model of mZP deals with oscillatory phenomenon of ZP structures as a whole.

Other biomechanical models of fertilization consider maximum pressure that single molecule generates on ZP surface as a necessary bio-mechanical condition for sperm penetration [8].

The oscillatory spherical net model of mZP assumes that fertilization is possible when ZP is in favorable oscillatory condition for fertilization-when majority of molecules oscillate in multifrequency regimes without energy dissipation (in linear elastic model) or with fractional order energy dissipations (in fractional order model) [9, 10]. The assumption of this model is that initial sperm penetration is possible when at least one sperm cell oscillates in resonance with ZP.

But neither the previous mechanical models of ZP based on the assumption that the ZP behaves as an ideal elastic body [8], as well as single-oscillator model [6,7], which includes an ideal elastic bonds between ZP molecules can not explain the visco-elastic properties of ZP in response to the pressure that were obtained in experiments [1,2].

Using finite element method (FEM) analysis it is possible to model a whole oocyte as 3D object [11, Hedrih & Banić under review] and analyse the oscillatory behaviour of whole oocyte. Using FEM analysis in numerical experiments (Hedrih & Banić, under review) has revealed that ZP behaves as visco-elastic body in initial sperm-ZP interaction. The authors suggested oscillations of relaxations as possible mechanism of sperm penetration trough ZP.

The theoretical consideration of the energy of bounds between ZP molecules and the mechanical force that can generate a single sperm [12] come to the idea that a possible mechanism of sperm penetration in this case, is the stress relaxation or ZP must be in the liquid state.

" The calculated force exerted by individual sperm is too small to permit the rupture of any but the weakest of secondary chemical bonds. Mechanical progress through the zona must rely on stress relaxation in a viscoelastic medium. The known properties of the zona appear to be consistent with such a mechanism of penetration "[12].

One-layered and multi-layered net will have different oscillatory behaviour in modelling process.

The mechanism of sperm penetration trough multi –layered net will be more complex. Some of the sperm cells will stay trapped in the outer layer while only one will reach inner layer and pass trough. Under what mechanical conditions is this mechanism possible?

In this modelling approach it is possible that maximum sperm generating pressure that could potentially rupture the ZP filaments is not the mechanism of sperm penetration but smallest resistant forces of ZP that is generated in response to mechanical influence of sperm cell [2, Hedrih & Banić under review].

The goal of this work is to create a multi-layered oscillatory model of mZP that could better describe oscillatory behavior of real biological system. The basic idea is to explain the sperm ZP penetration mechanism using double-layered mZP oscillatory model.

2. Multi-layer oscillatory spherical net model of mZP

The basic concept of double-layered mZP oscillatory model will be presented.

Double-layered mZP oscillatory spherical models are improved one-layered oscillatory spherical net model [6,7]. Two new variables were included into the model: double-layered network and visko-elastic properties between its constructive elements.

In double-layered mZP oscillatory model interconnections between ZP glycoproteins have fractional order properties. In this way oscillatory behavior of ZP from pure elastic to viscous is covered. Double-layered model with fractional order properties has advantage under one-layered model with ideally elastic properties because it can explain change in ZP mechanical properties before,in a course and after fertilization [1,2].

Molecules are interconnected with standard light fractional order visco-elastic element defined by constitutive relation force - elongation expressed by fractional order derivatives.

The other advantage is that model is double-layered that resembles better to the real system [3, 4]. On SEM analysis outer ZP layer is with larger and inner with smaller fenestrations [3,4].

Since we do not know how filaments are arranged in layers, as well as what is the relationship of filaments in layers, we propose different variants of relations of two layers in the network.

Two identical nets can be placed one on the other in different ways.
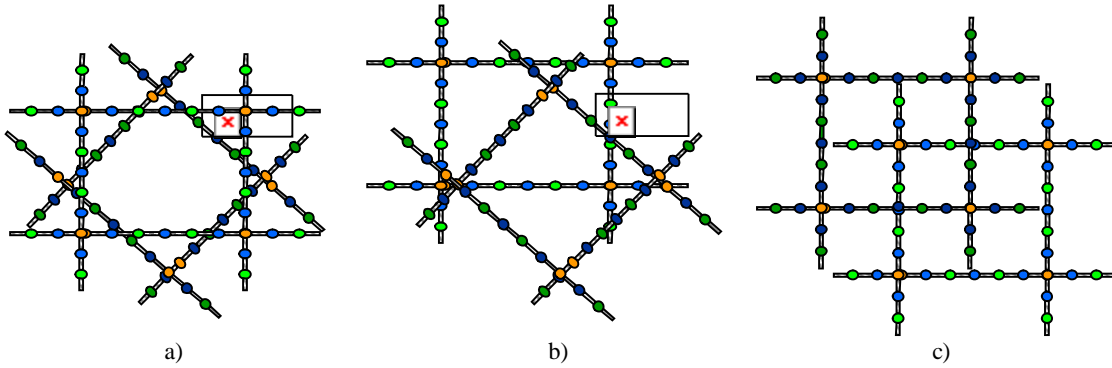

a)   b)   c)

Fig. 1. Some variants of possible ways of nets overlapping in fractional order double-layered spherical net model of mZP- a representative segment.

Assuming that both layers have identical regular geometry and structure, examples of double-layered oscillatory networks are presented on Fig 1. Each molecule is interconnected with two neighboring molecules and with ZP surface with standard light fractional order visco-elastic elements. At places when upper and lower net are overlapped (not molecules or ZP2 molecules as on -Fig 1c), nets are interconnected by these molecules with standard light fractional order visco-elastic element.

On a Fig 2 and 3 are presented case when both layers have same composition, lower net is rotated for angle of 45° compared to upper net overlapping the knot molecules at one diagonal.

Sides of the square of upper spherical net corespond to the diagonal of the square of the lower spherical net. In the undeformed state interconection lenght are longer in upper than in the lower net making that fenestrations of upper net are larger than those of the lower. This assumtion coresponds to experimental results [3,4]. In oscillatory state this reactions depend of displacements of knot molecules respecting that oscilatorry state of double-layered net.

Each layer consists of circular and meridian chains that are orthogonal. For analysis of oscillatory behavior of double –layered ZP net we are going to use the small and representative segment of the net presented on Fig 2 and 3. The double –layered ZP segment consists of two identical segments-each segment has two circular and two meridian chains, but in positions of the angle of 45°degree (in upper to lower pairs-chains directions). Each chain consists of 11 molecules arrange on specific manner [6, 7] and interconnected by standard light fractional order element.

Chain in upper net that interconnect knot molecules along diagonal can be considered as a chain in radial direction and with two degrees of freedom.

The oscillatory behavior of each chain with eleven degrees of freedom is described by the system of eleventh ordinary fractional order differential equations expressed along molecule displacements in chain direction. For radial displacement of each molecule there is a fractional

order differential equation. For knot molecules there are three component displacements in circular, meridian and radial directions.
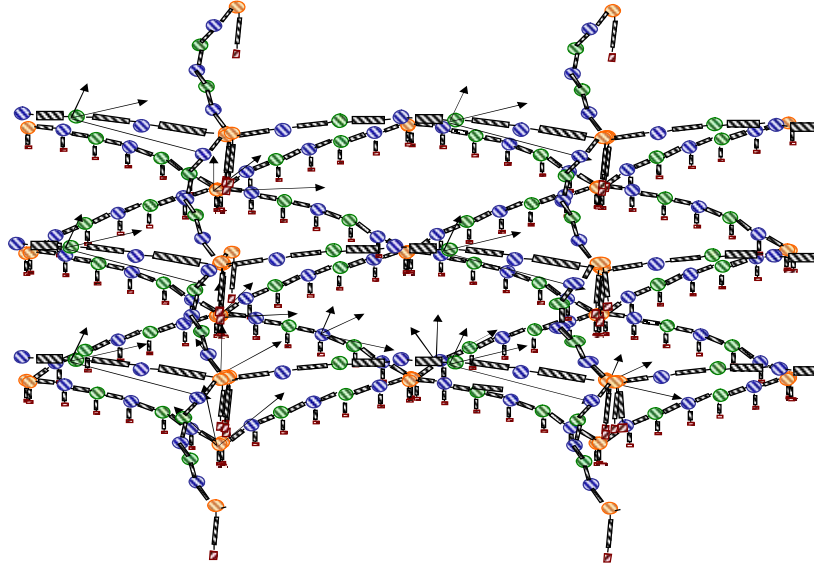

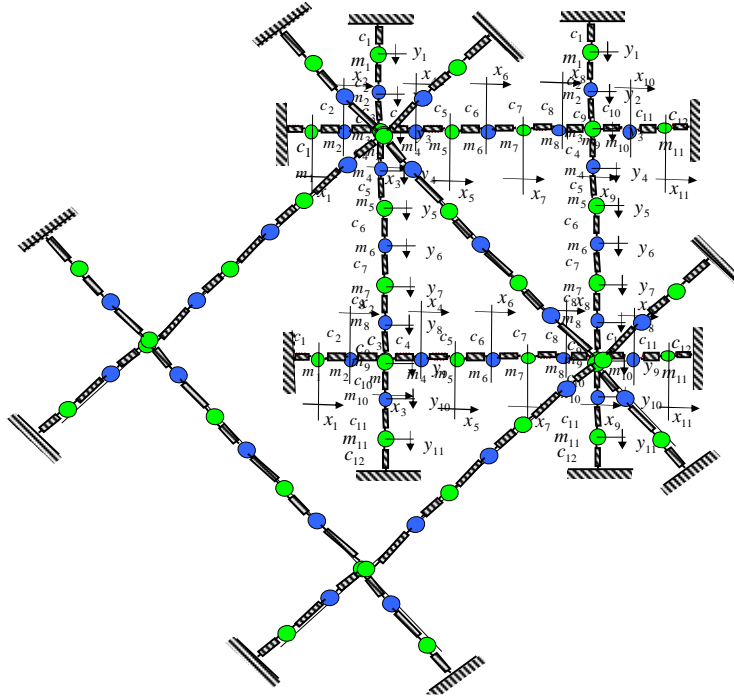Fig. 2. Part of double-laired net in oscillatory spherical net model of mZP

Fig. 3. Part of double-laired net in oscillatory spherical net model of mZP with fractional properties-possible arrangement

For calculating resultant displacements of knot molecules that interconnect two nets, it must be taken into account that directions of chains in upper and lower net are at an angle of 45° degrees relative to each other. Each two orthogonal direction along two arbitrary orthogonal circles along sphere surface are circular and meridian, and chains are in main directions. This is due to central

symmetry of spherical surface net. Relying on this fact a general model of a chain can be considered as well as analysis of kinetic parameters and eigen main fractional mode oscillations. Taking into account space positions of each molecule component displacement it is possible to make combinations and calculate resultant displacements.

System of ordinary fractional order differential equations for fractional order oscillations of chains with 11 molecules (material particles) of representative segment of *lower (inner)* spherical net are in the following form (see Fig. 3) :

* for molecules in chains in circular direction:

$$m_{k,j}\ddot{u}_{k,j}(t)+c_{0(k-1,k),j}\left[u_{k,j}(t)-u_{k-1,j}(t)\right]-c_{0(k,k+1),j}\left[u_{k+1,j}(t)-u_{k,j}(t)\right]+c_{0\leq\alpha\leq1(k-1,k),j}\mathsf{D}_t^{\alpha}\left[u_{k,j}(t)-u_{k-1,j}(t)\right]-$$
$$-c_{0\leq\alpha\leq(k,k+1),j}\mathsf{D}_t^{\alpha}\left[u_{k+1,j}(t)-u_{k,j}(t)\right]=0$$

$k=1,2,3,...,11$, $j=1,3$, $0\leq\alpha\leq1$ \hfill (1)

* for molecules in chains in meridian direction:

$$m_{k,j}\ddot{v}_{k,j}(t)+c_{0(k-1,k),j}\left[v_{k,j}(t)-v_{k-1,j}(t)\right]-c_{0(k,k+1),j}\left[v_{k+1,j}(t)-v_{k,j}(t)\right]+c_{0\leq\alpha\leq1(k-1,k),j}\mathsf{D}_t^{\alpha}\left[v_{k,j}(t)-v_{k-1,j}(t)\right]-$$
$$-c_{0\leq\alpha\leq(k,k+1),j}\mathsf{D}_t^{\alpha}\left[v_{k+1,j}(t)-v_{k,j}(t)\right]=0$$

$k=1,2,3,...,11$, $j=2,4$, $0\leq\alpha\leq1$ \hfill (2)

* **f**or all molecules of inner net except knot molecules and for knot molecules that are not interconnected to the upper (outer) net knot molecule:

$$\tilde{m}_{k,j}\ddot{w}_{k,j}(t)+\tilde{c}_{0(k,j),j}w_{k,j}(t)+c_{0\leq\alpha\leq1(k,j)}\mathsf{D}_t^{\alpha}\left[w_{k,j}(t)\right]=0 \quad k,j=1,2,3,...,11, \quad k\neq3, \quad k\neq9, \ 0\leq\alpha\leq1 \quad (3)$$

* for knot molecules that interconnect inner and upper net and are connected to the ZP surface by standard light fractional order elements:

$$\tilde{m}_{k,j}\ddot{w}_{k,j}(t)+\tilde{c}_{00(k,j),j}w_{k,j}(t)-\tilde{c}_{0(k,j),j}\left[\tilde{w}_{k,j}(t)-w_{k,j}(t)\right]-\tilde{c}_{0\leq\alpha\leq1(k,j)}\mathsf{D}_t^{\alpha}\left[\tilde{w}_{k,j}(t)-w_{k,j}(t)\right]+$$
$$+\tilde{c}_{0.0\leq\alpha\leq1(k,j)}\mathsf{D}_t^{\alpha}\left[w_{k,j}(t)\right]=0$$

$k=3$, $or/and \ k=9$, $j=3$, $or/and \ j=9$, $0\leq\alpha\leq1$ \hfill (4)

$$\tilde{\tilde{m}}_{k,j}\ddot{\tilde{w}}_{k,j}(t)+\tilde{c}_{0(k,j),j}\left[\tilde{w}_{k,j}(t)-w_{k,j}(t)\right]+\tilde{c}_{0\leq\alpha\leq1(k,j)}\mathsf{D}_t^{\alpha}\left[\tilde{w}_{k,j}(t)-w_{k,j}(t)\right]=0$$

$k=3$, $or/and \ k=9$, $j=3$, $or/and \ j=9$, $0\leq\alpha\leq1$ \hfill (5)

System of ordinary fractional order differential equations for fractional order oscillations of chains with 11 molecules (material particles) of representative segment of *upper* (outer) spherical net are in the following form (see Fig. 3) :

* for molecules in chains in circular direction (at angle of 45˚ compared to lower net circular chain):

$$m_{k,j}\ddot{\tilde{u}}_{k,j}(t)+c_{0(k-1,k),j}\left[\tilde{u}_{k,j}(t)-\tilde{u}_{k-1,j}(t)\right]-c_{0(k,k+1),j}\left[\tilde{u}_{k+1,j}(t)-\tilde{u}_{k,j}(t)\right]+c_{0\leq\alpha\leq1(k-1,k),j}\mathsf{D}_t^{\alpha}\left[\tilde{u}_{k,j}(t)-\tilde{u}_{k-1,j}(t)\right]-$$
$$-c_{0\leq\alpha\leq(k,k+1),j}\mathsf{D}_t^{\alpha}\left[\tilde{u}_{k+1,j}(t)-\tilde{u}_{k,j}(t)\right]=0$$

$k=1,2,3,...,11$, $j=1,3$, $0\leq\alpha\leq1$ \hfill (6)

* for molecules in chains in meridian direction (at angle of 45˚ compared to lower net meridian chain):

$$m_{k,j}\ddot{\tilde{v}}_{k,j}(t)+c_{0(k-1,k),j}\left[\tilde{v}_{k,j}(t)-\tilde{v}_{k-1,j}(t)\right]-c_{0(k,k+1),j}\left[\tilde{v}_{k+1,j}(t)-\tilde{v}_{k,j}(t)\right]+c_{0\leq\alpha\leq1(k-1,k),j}\mathsf{D}_t^{\alpha}\left[\tilde{v}_{k,j}(t)-\tilde{v}_{k-1,j}(t)\right]-$$
$$-c_{0\leq\alpha\leq(k,k+1),j}\mathsf{D}_t^{\alpha}\left[\tilde{v}_{k+1,j}(t)-\tilde{v}_{k,j}(t)\right]=0$$

$k=1,2,3,...,11$, $j=2,4$, $0\leq\alpha\leq1$ \hfill (7)

where $u_{k,j}(t)$, $v_{k,j}(t)$ and $w_{k,j}(t)$ are molecule component displacements in circular, meridian and radial delectations representative segment of lower net, $\tilde{u}_{k,j}(t)$, $\tilde{v}_{k,j}(t)$ and $\tilde{w}_{k,j}(t)$ are molecule component displacements in circular, meridian and radial delectations of characteristic, representative segment of upper net, $\mathsf{D}_t^\alpha[\bullet]$ is fractional order differential operator of the αth derivative with respect to time t in the following form:

$$\mathsf{D}_t^\alpha[\bullet] = \frac{d^\alpha[\bullet]}{dt^\alpha} = \frac{1}{\Gamma(1-\alpha)}\frac{d}{dt}\int_0^t \frac{[\bullet]}{(t-\tau)^\alpha}d\tau \tag{8}$$

$\Gamma(1-\alpha)$ is Euler Gama function, and $c_{0(k,k+1)}$ and $c_{\alpha(k,k+1)}$ are rigidity coefficients – momentary (expressing ideal elastic properties) and prolonged one (expressing fractional order dissipation properties), α is a rational number between 0 and 1, $0 < \alpha < 1$, expressing elastic and fractional order dissipation properties of standard light fractional order element.

All molecules in double – layered oscillatory net model are connected to the ZP surface in radial direction. Differential fractional order equations describing fractional order oscillations components in radial directions of molecules in upper and inner representative segment of the model are same as in (1) and (3).

Molecules in double-layered ZP net oscillate in fractional order oscillatory regimes containing each eleventh eigen fractional order modes when they are disturbed as perturbation from equilibrium static positions to dynamic state induced by initial velocities of molecules induced by spermatozoa impacts to the corresponding molecule. That occurs when initial velocities are applied to inner/outer layer of double-layered fractional order ZP net in circular/meridian directions.

Component eigen oscillatory modes of molecules in oscillatory fractional order regime in form of particular solutions like cosine $T_{s,\cos}(t,\alpha)$ and like sine $T_{s,\sin}(t,\alpha)$ [9, 10, 13] are:

$$T_{s,\cos}(t,\alpha) = \sum_{k=0}^{\infty}(-1)^k \omega_{(\alpha)s}^{2k} t^{2k} \sum_{m=0}^{k}\binom{k}{m}\frac{\omega_{(\alpha)s}^{-2m}t^{-\alpha m}}{\omega_s^{2m}\Gamma(2k+1-\alpha m)}, \quad s=1,2,3,...,11, 0 < \alpha < 1 \tag{9}$$

$$T_{s,\sin}(t,\alpha) = \sum_{k=0}^{\infty}(-1)^k \omega_{(\alpha)s}^{2k} t^{2k+1} \sum_{m=0}^{k}\binom{k}{m}\frac{\omega_{(\alpha)s}^{-2m}t^{-\alpha m}}{\omega_s^{2m}\Gamma(2k+2-\alpha m)}, \quad s=1,2,3,...,11, \ 0 < \alpha < 1 \tag{10}$$

where ω_s, $s=1,2,3,...,11$ and $\omega_{(\alpha)s}$, $s=1,2,3,...,11, 0<\alpha<1$ eigen characteristic numbers of fractional order chains, ω_s, $s=1,2,3,...,11$ are equal to eigen circular frequencies of corresponding linear elastic chain with same degrees of freedom.

When component displacements of knot molecules that interconnect inner and outer fractional order ZP net are calculate for comparison in a coordinate system it is necessary to take that circular and meridian directions in inner and outer nets are with respect to one and other at an angle of 45° degrees. Component and resultant displacements of this knot molecules must be take into account in a common coordinate system. If the reference coordinate system consists of radial and circular directions of knot molecules from the inner representative segment of fractional order ZP net than, component displacements of knot molecules in circular and meridian directions are:

* for knot molecule $\tilde{m}_{k,j}$ of inner part of double-layered fractional order ZP net

    - in circular displacement is $u_{k,j,in}(t) = u_{k,j}(t)$ and

    - in meridian displacement $v_{k,j,in}(t) = v_{k,j}(t)$

for $k=3$, $or/and\ k=9$, $j=3$, $or/and\ j=9$, $0 \le \alpha \le 1$.

* for knot molecule $\tilde{\tilde{m}}_{k,j}$ of outer part of double-layered fractional order ZP net

    - in circular displacement (in reference coordinate system) is:

$$\tilde{u}_{k,j,out}(t) = \frac{\sqrt{2}}{2}\left[\tilde{u}_{k,j}(t) + \tilde{v}_{k,j}(t)\right] \tag{11}$$

and

    - in meridian displacement (in reference coordinate system) is:

$$\tilde{v}_{k,j,spolj}(t) = \frac{\sqrt{2}}{2}\left[\tilde{u}_{k,j}(t) - \tilde{v}_{k,j}(t)\right] \tag{12}$$

for $k = 3$, $or / and\ k = 9$, $j = 3$, $or / and\ j = 9$, $0 \le \alpha \le 1$, because of $\cos 45° = \sin 45° = \dfrac{\sqrt{2}}{2}$

Using double-layered oscillatory fractional order ZP net it is possible to explain the mechanism of sperm penetration trough ZP. Sperm cell have the more demandable task to pass trough more dense environment than in the case of one layered net on easiest way. I*n vitro* [14] and numerical [7] experiments have been found that sperm create an oblique path trough ZP. Double-layered oscillatory fractional order ZP net model gave basis that possible mechanism of sperm penetration trough ZP are oscillations of relaxations.

3. Conclusion

In this paper we proposed double-layered models with fractional order properties that could explain transition in mZP behavior before and after fertilization. Beside it is suitable for explaining mechanism of penetration of spermatozoa trough ZP thickness on a basis of oscillations of relaxations. Besides this double - layered model could serve as basis for next more complex model of mZP where mZP will be considered as an oscillating gel. Modeling mZP as an oscillating gel it will be possible to investigate nonlinear dynamical phenomena that can arise from a coupling of mechanical and chemical energy"[15].

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