

# Fractional calculus evaluation of hyaluronic acid crosslinking in a nanoscopic part of articular cartilage model system

P. Beldowski<sup>1</sup>, P. Weber<sup>2</sup>, T. De Leon<sup>3</sup>, W. K. Auge II<sup>4</sup>, A. Gadomski<sup>5</sup>

<sup>1</sup> P. Beldowski, University of Science and Technology, Bydgoszcz, Poland, [piobel000@utp.edu.pl](mailto:piobel000@utp.edu.pl)

<sup>2</sup> P. Weber, Gdańsk University of Technology, Gdańsk, Poland, [pioweber@pg.edu.pl](mailto:pioweber@pg.edu.pl)

<sup>3</sup> T. De Leon, Delaware State University, Dover, Delaware, USA, [tdeleon15@students.desu.edu](mailto:tdeleon15@students.desu.edu)

<sup>4</sup> W. K. Auge II, NuOrtho, Boston, Massachusetts USA, [nnmoc@aol.com](mailto:nnmoc@aol.com)

<sup>5</sup> A. Gadomski, University of Science and Technology, Bydgoszcz, Poland, [agad@utp.edu.pl](mailto:agad@utp.edu.pl)

## Abstract

This work presents a study of the mechanism of physical crosslinking of hyaluronic acid in the presence of common phospholipids in synovial joint organ systems. Molecular dynamic simulations have been executed to understand the formation of hyaluronan networks at various phospholipid concentrations. The results of the simulations suggest that the mechanisms exhibit subdiffusion characteristics. Transportation quantities derive as a function of time during numerical calculations of mean square displacement, and observations of sublinear growth were noted. Coarse-grained models are deployed to obtain a mathematical description where a random walker and several subdiffusion schemes of its motion describe the models. The findings of this study may establish mechanisms of biopolymer network formations in normal and pathologic synovial fluid and help elucidate the mechanism of facilitated AC biolubrication.

**Keywords:** Hyaluronic acid, Molecular dynamics, Lubrication, Fractional calculus

## 1 Introduction

Hyaluronic acid is a major component in many systems in the body, and serves an important role in each of those systems. This study focuses on hyaluronic acid and its role in the complex synovial joint system and how it is influenced by common phospholipids [19, 11, 9, 20, 4]. In a healthy AC synovial joint system, hyaluronic acid contributes to viscoelasticity, lubrication, and overall joint support [1]. Hyaluronic acid is beneficial to the system when it is allowed to crosslink into a supramolecular structure needed for facilitated lubrication [5]. As changes in physiology occur, the properties of the hyaluronic acid in the system change [8]. In pathogenesis, a number of changes in the system occur, which ultimately alters the efficacy of HA. When common phospholipids interact with the HA, physical crosslinking is inhibited and the viscoelasticity of the synovial fluid decreases [17]. Using in silica experiments on the YASARA software program [21], models were designed to imitate a nanoscopic section of the fluid filled synovial joint. This occurrence is examined by the findings of this study and indicate that the traditional treatment for osteoarthritic joints of intra-articular HA injections is ineffective for lubrication, and other options should be explored.

Hyaluronic acid and phospholipids are both very important in the biolubrication mechanism of synovial joint organ systems. Both molecules are considered in non-surgical treatment of osteoarthritis via intra-articular injections in the clinical field and provide short term relief, but are

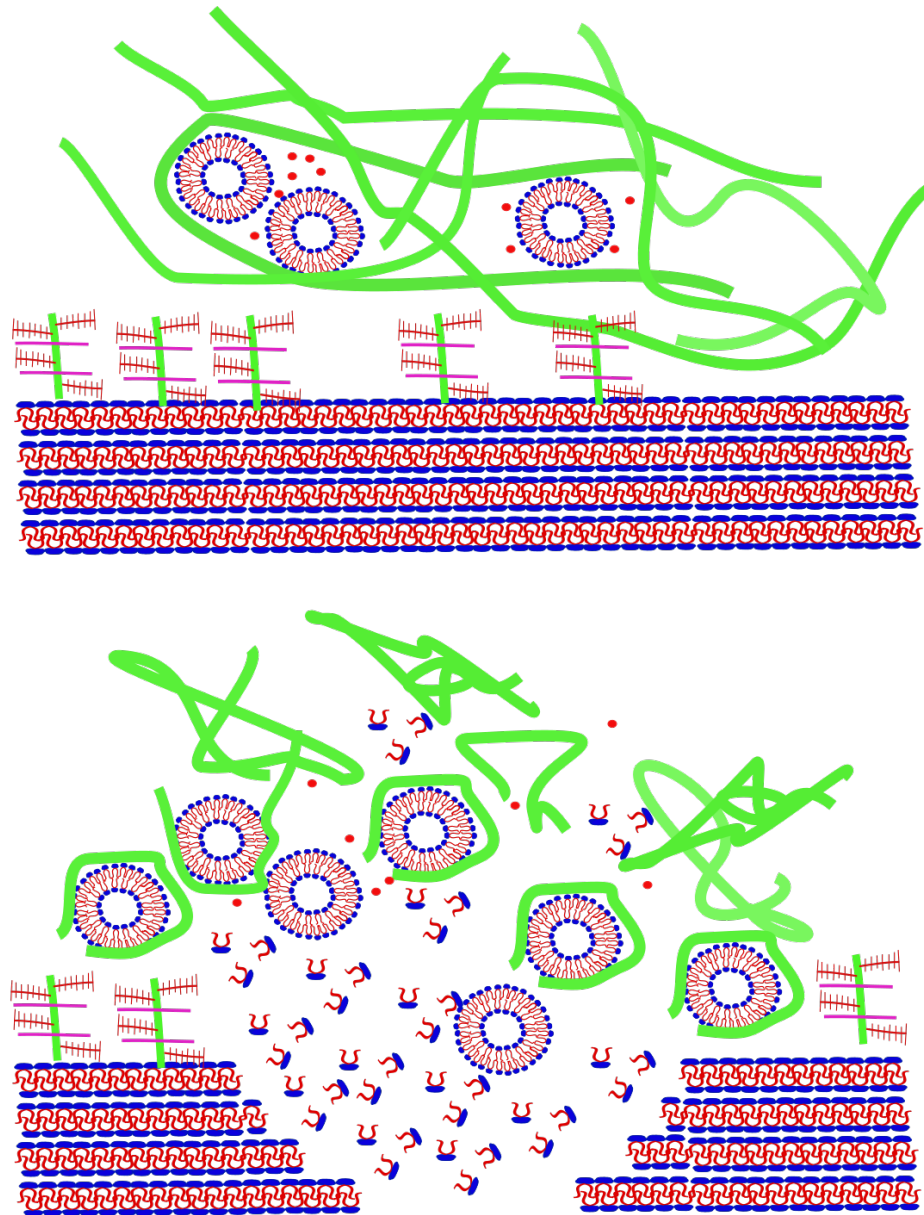


Figure 1: The artistic depiction of articular cartilage at different stages of functionality. The top part present the healthy (normal) physiological conditions. Lower part present the abnormal (pathological conditions). Degeneration of AC surface leads to increase of phospholipids and lowers crosslinking mechanism.

not permanent solutions. As depicted in Figure 1, the top image presents a healthy system with crosslinked HA while the bottom image depicts a system in which the green hyaluronic acid chains are not able to crosslink due to the increased levels of phospholipids.

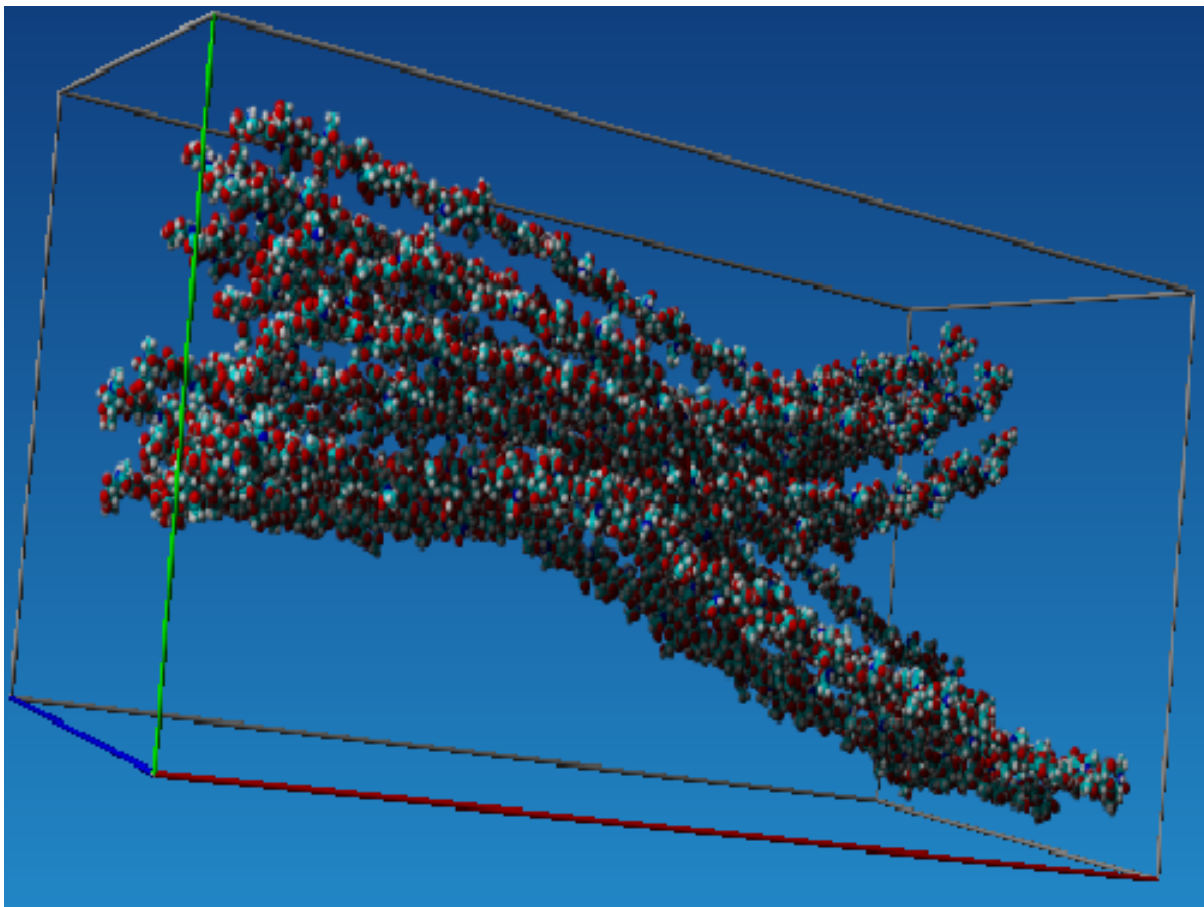


Figure 2: The initial structures of systems of interest. Water molecules as well as lipids are not presented. Red dots represent oxygen atoms, blue - carbon and white hydrogen.

## 2 Methods

The molecular dynamics simulation method has been used to look at the interactions between two components of synovial fluid. The system is composed of 16 chains of hyaluronic acid with a length of 25 nm (10 kDa) in the extended form and parallel to each other. The simulation box is filled with saline solution 0.9% NaCl to obtain 5% solution of HA, and then the different numbers of lipids (DPPC) are added to obtain concentration of HA and lipids at ratios between components denoted as  $k_{PL:HA} = \frac{c_{PL}}{c_{HA}}$ , where  $c_{PL}$  and  $c_{HA}$  represent concentration of PL and HA respectively. The modeled lipid DPPC (dipalmitoylphosphatidocholine) has been chosen because of its highest concentration among other lipids in synovial fluid [7]. The simulation that ran for 10ns was performed at constant temperature of 310K at constant  $pH = 7$ . Figure 2 presents the initial structure of a system without water and lipids visualisation.

Assisted Model Building with Energy Refinement (AMBER) force field has been employed to mimic interactions between molecules, due to its universality and good description of interaction. YASARA structure package was used for molecular dynamics simulations. To describe a process of crosslinking of HA this work focused on two main phenomena when physical crosslinking occurs, namely hydrophobicity of the network and number of contacts reflecting how close chains are to

each other.

The number of hydrophobic interactions between hydrophobic atoms is calculated by number of carbon atoms of groups close to other carbon based groups with a distance less than  $0.5nm$ . Radius of gyration  $R_g$  is a measure:

$$\vec{R}_g = \sqrt{\frac{1}{N} \sum_{i=1}^N (\vec{R}_i - \vec{C})^2} \quad (1)$$

Here  $\vec{R}_i$  stand for position of each atom in the system and  $\vec{C}$  is a center of a mass. Solvent accessible surface, a measure describing penetration of water molecules into molecule, consists of all the points that the center of the water probe (i.e. the nucleus of the oxygen atom in the water molecule) can reach while rolling over the solute. The shortest possible distance between the water oxygen nucleus and a solute atom is simply the sum of the Van der Waals radii of the solute atom and the water probe was calculated with method described by [14]. The number of contacts between atom lists those pairs of selected units that are closer than the specified cutoff parameter which in our case is  $0.5nm$ .

### 3 Results

Figure 3 presents the final structures of system at different PL concentrations with increasing number of phospholipids from top to bottom. Evolution of  $R_g$  (Figure 4) shows that there is

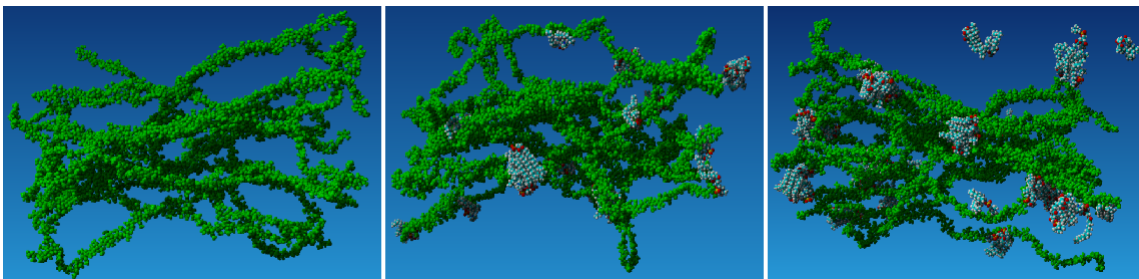


Figure 3: The final structures of systems of interest. The HA has been colored green for better visualisation, color of atoms building lipids are colored in the same fashion as in the Figure 2. From left to right the concentration of lipids increases.

no significant difference between all three cases. The reason behind these is low cmc(critical micelle concentration) of DPPC lipids, where they tend to create micelles rather than interact with HA. Hence the penetration of HA network is low. The result is different that one obtained previously [17], however this is a result of initial conditions that imitates well established network. As presented in Figure 5 water molecules penetration is increased with PLs concentration. Figure 6 shows an apparent mechanism of repelling water from network in absence of lipids. This means that the presence of lipids help water molecules to better penetrate system. This means that the denser, stronger network is created in absence or low concentration of lipids. Figure 7 shows that mechanism from perspective of overall number of intramolecular contacts inside network.

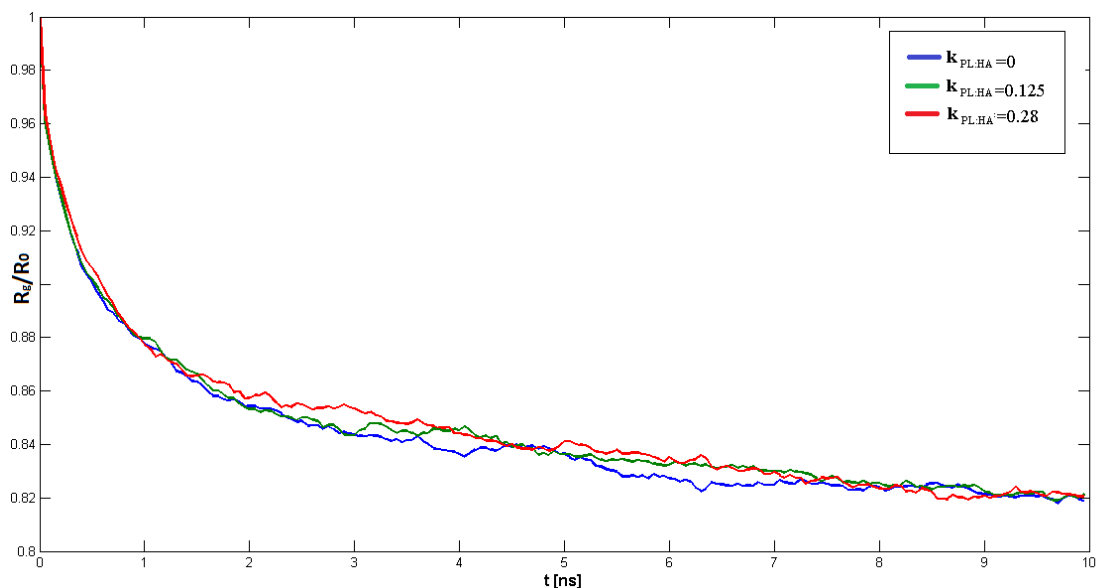


Figure 4: Relative radius of gyration in function of time. All the cases are described in legend of a plot.

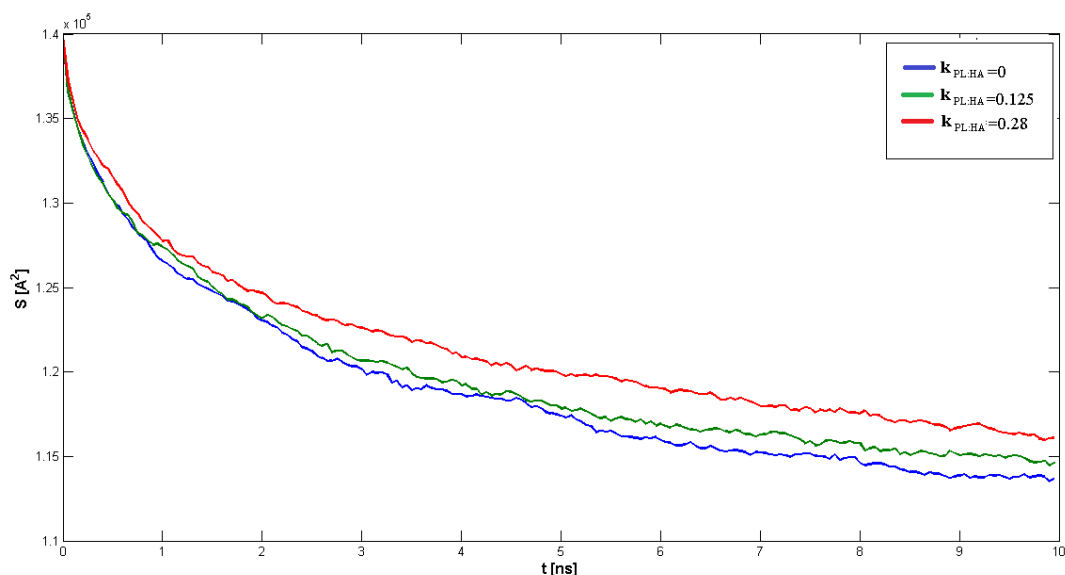


Figure 5: Solvent accessible surface in function of time. All the cases are described in legend of a plot.

## 4 Fractional model of phenomenon

Polymeric system usually exhibit anomalous dynamics, where the mean-square displacement of a single monomer increases as  $t^\alpha$ ,  $0 < \alpha < 1$  until to a certain time limit  $\tau$ . It means that until time  $\tau$  the dynamics has subdiffusive character. After  $\tau$  the process becomes diffusive, where mean-square displacement of a single monomer is proportional to time [10].



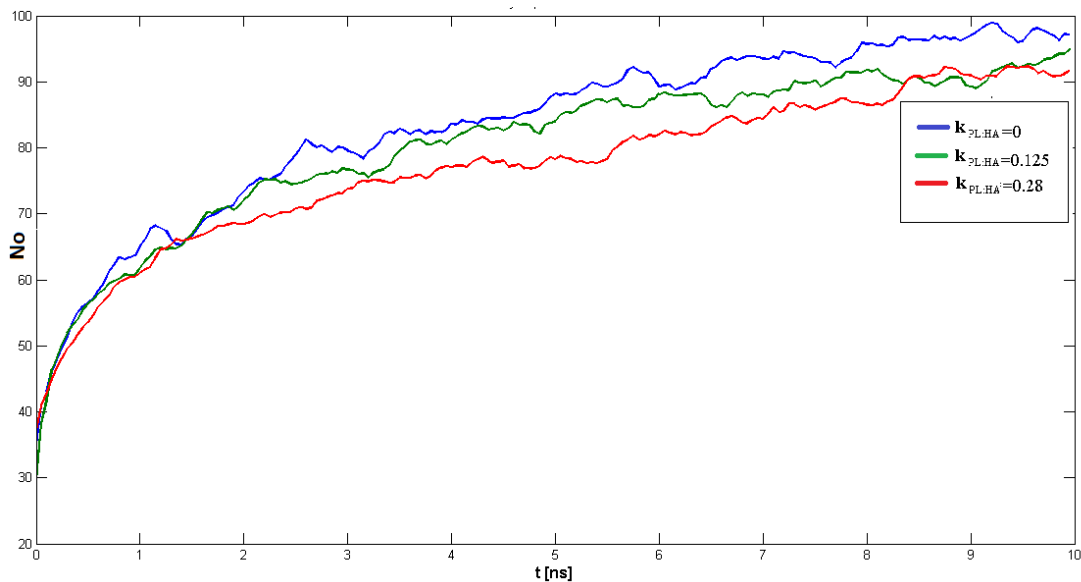


Figure 6: Number of hydrophobic interactions between atoms in function of time. All the cases are described in legend of a plot.

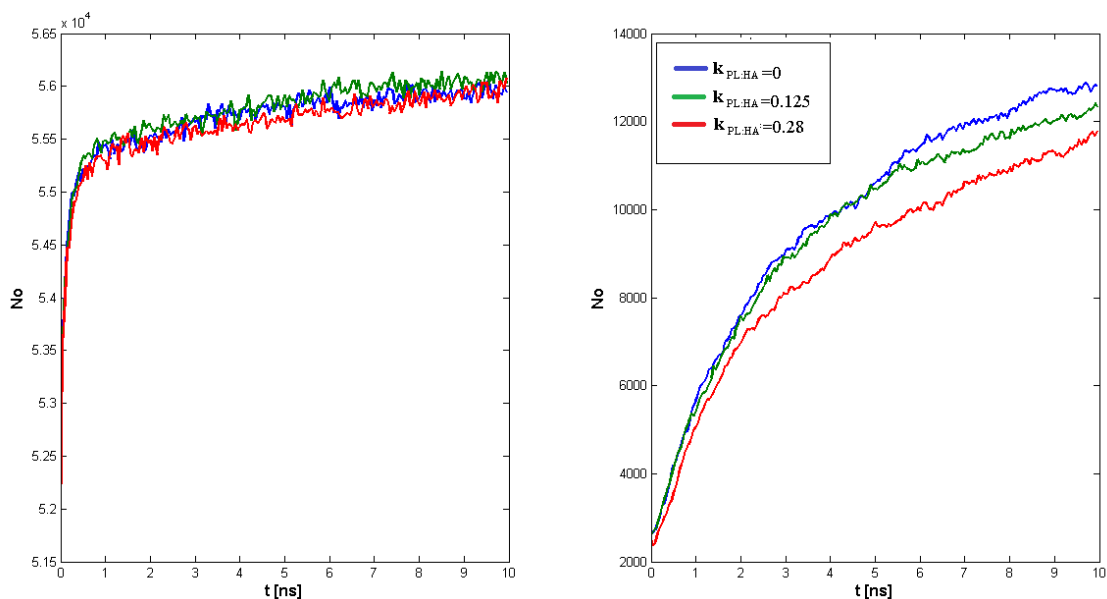


Figure 7: Contacts between atoms in function of time. All the cases are described in legend of a plot. On the left hand side one can see the results for intra-molecular, on the right hand side inter-molecular contacts between HA chains.

There is many models, that explain such observations [2]. This work uses various ideas coming from several of them. Example of such a model is the self-avoiding Rouse model [10], that gives an incomplete explanation about the observations. This model is based on assumptions of both: Rouse model [15] and self-avoiding walk model. The polymeric macromolecule is divided into smaller parts called submolecules. Submolecules can be treated as a rigid bead. In the most simple formulation of the Rouse model there is assumption that in polymeric dynamics neglect

interactions between the submolecules that perform gaussian motion. Motions of submolecules are independent between each other. This is a drawback of this theory. Self avoiding walk completes the Rouse theory, but not fully. There is introduced the fact, that submolecules (bead) of polymer can't occupy the same point in space. More advance models assume that between submolecules there are interactions (for example: the Zimm model). But our results suggest to use another model to describe them. We propose to use ideas presented in [3].

Polymeric systems are large macromolecules that have several length - scale dependent relaxation times. The longest one of them is called the terminal relaxation time  $\tau$ . Time  $\tau$  manifested in the decay of the polymer's end-to-end vector correlation function. Using idea of Rouse model one can divide HA molecule on submolecules and we follow their dynamics. Mean square displacement of a single submolecule of a polymer, for  $t < \tau$ , behave as  $t^\alpha$ . For  $t > \tau$  process is proportional to time. So one can conclude that the dynamics of a tagged submolecule in a polymer chain must be anomalous till the terminal relaxation time  $\tau$ .

To compare simulations results with theoretical predictions we have plotted mean-square displacement of a single submolecule in the function of time in logarithmic - logarithmic scale. This results are presented on the Figure 8. One can see that dependency of mean-square displacement

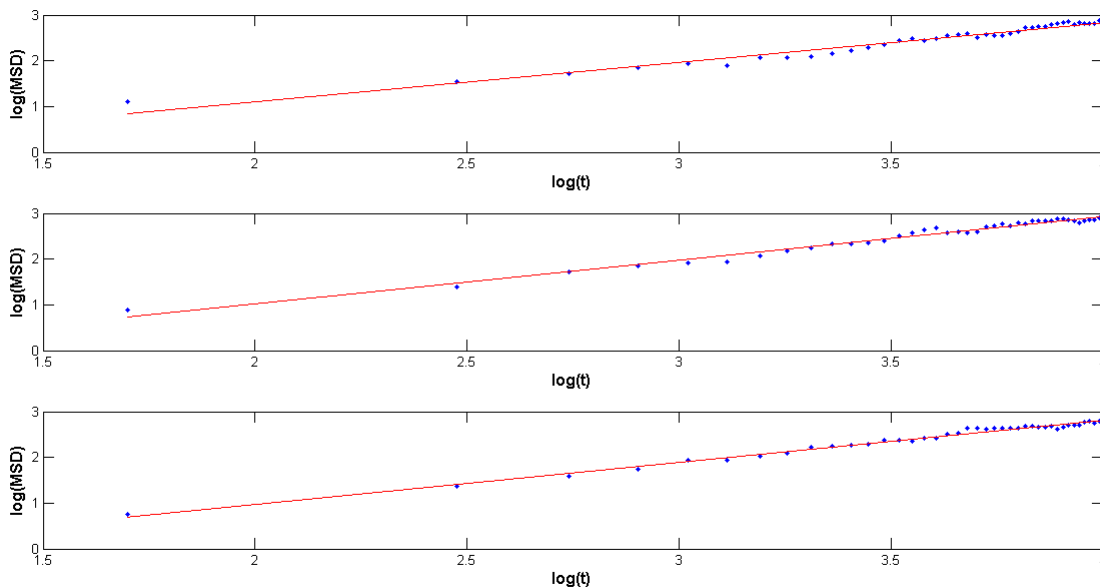


Figure 8: MSD in loglog scale top row represent organised structure while lower row show random structure. Top to bottom one can see a system with growing concentration of lipids. Parameters of the fit are presented in Table 1

of a single submolecule from time as power function, until time  $\tau$  is well fulfilled. Goodness of fit of a model to data from simulation is measured by using coefficient of determination  $R^2$ . In the table one can see that  $R^2$  has very high values. It means that assumption about power dependency mean-square displacement of a single submolecule from time is well fulfilled.

Taking into account values of  $\alpha$  parameter and its dependency from concentration we propose

Table 1: Parameters of fitting with a linear function to MSD

Case	HA	HA+24	HA+56
$R^2$	0.9640	0.9801	0.9884
$\alpha$	0.8621	0.9537	0.9192

to describe process in the framework of a model presented in [3]. In the article there is explanation of behaviour of fully hydrated dipalmitoylphosphatidylcholine membrane during the gel-to-subgel phase transformation process. Author argued that description of it is a problem of time scale. This work uses this idea to presented here results of simulations.

In dynamics of HA molecule one can distinguish two phases. First when one observes anomalous dynamics and the second when the system achieves its final structure and motion of its part is pure random. There is of course not only one system but statistical ensemble of such molecular systems. Properties of single polymeric molecule generally is determined by hydrophobic and hydrophilic forces. So the properties of statistical ensemble of molecule is also determined by them. To proposed model that describe the ensemble one can assume that there are some number of hydrophobic forces  $N_f$  and some number of hydrophilic forces  $N_h$ , both depend on time. One assumes that numbers of all forces acting on a molecule is constant:

$$N = N_f(t) + N_h(t). \quad (2)$$

Taking into account results of simulations of hydrophobic contacts we propose to use in description of  $N_f(t)$  after time  $\tau$  equation in the form:

$$M \frac{d}{dt} N_f(t) = (N - N_f(t)) \quad (3)$$

This equation has solution in the form:

$$N_f(t) = N_f(0)e^{-At} + N(1 - e^{-At}) \quad (4)$$

where  $N_f(0)$  represents initial number of contacts. But before time  $\tau$  one can observe processes, that causes unnormal kinetic. This part of the process can be described by formula:

$${}_0^{RL}D_t^\alpha [N_f(t)] = A_\alpha(N - N_f(t)), \quad (5)$$

where  ${}_0D_t^\alpha [\cdot]$  is a Riemann-Liouville derivative defined, for  $0 < \alpha < 1$ , by formula [12, 16]

$${}_0^{RL}D_t^\alpha [f(t)] = \frac{1}{\Gamma(1-\alpha)} \frac{d}{dt} \int_0^t \frac{f(\tau)}{(t-\tau)^\alpha} d\tau. \quad (6)$$

To solve this equation one can use Caputo derivative defined, for  $0 < \alpha < 1$ , by:

$${}_0^CD_t^\alpha [f(t)] = \frac{1}{\Gamma(1-\alpha)} \int_0^t \frac{1}{(t-\tau)^\alpha} \frac{df(\tau)}{d\tau} d\tau \quad (7)$$

and relationship between both kind of derivatives [12, 13]

$${}_0^{RL}D_t^\alpha [f(t)] = {}_a^CD_t^\alpha [f(t)] + \frac{t^{-\alpha}}{\Gamma(1-\alpha)} f(0). \quad (8)$$



Using the last relation one can write proposed equation in the following way:

$${}_0^C D_t^\alpha [N_f(t)] + \frac{t^{-\alpha}}{\Gamma(1-\alpha)} N_f(0) = A_\alpha (N - N_f(t)). \quad (9)$$

Using the Laplace transform method to solve differential equation one can obtain following solution for proposed equation and initial condition  $N_f(0) = 0$ :

$$N_f(t) = A_\alpha N [1 - (E_{\alpha,1}(-A_\alpha t^\alpha) + (A_\alpha - 1) t^\alpha E_{\alpha,1+\alpha}(-A_\alpha t^\alpha))]. \quad (10)$$

Here, for  $x > 0$ ,  $\alpha > 0$  and  $\beta > 0$ , :

$$E_{\alpha,\beta}(x) = \sum_{k=0}^{\infty} \frac{x^k}{\Gamma(\beta + \alpha k)} \quad (11)$$

is a Mittag-Leffler function [6]. Transition from anomalous to normal kinetics is equivalent to replace fractional derivative to normal derivative.

## 5 Conclusions

There is well established connection between AC tribology and mechanisms of synovial joint organ system lubrication as governed in partial by HA:PL interaction [18, 1]. Presented results continues the description of system of interest as an interplay between HA network creation in various physiological conditions [8, 17]. This study further demonstrates that the increased concentration of PL connected to various physiological conditions have an influence on HA network creation.

Results show a mechanism of HA network creation/maintenance in a presence of phospholipids. Unlike in previous work on HA:PL interaction [17] there is no difference in geometrical parameters of a system, namely radius of gyration  $R_g$  (see Fig. 4). This is caused by initial conditions of the systems which meant to mimick the well established network of HA. Due to its hydrophilic nature HA interact rather within network than with lipids. There is however changed in interaction inside network. As presented in Figure 7 increase in PL concentration results in lower number of intermolecular contacts between HA chains. Final structures of simulations (Figure 3) show that PL can penetrate HA network and can create complexes of HA network micelle like structures. This can also be seen when looking at the behavior of water molecules. Figure 5 shows that HA without phospholipids create denser network repelling water molecules from the interior. This can also be seen in Figure 6, PL concentration cause in higher number of hydrophobic interactions between HA chains. This result is in good agreement with experimental results which show that PLs increase hydrophobicity of HA. Presented model of HA gel formation rationalize the obtained results in terms of fractional calculus. Process is governed by hydrophobic interactions therefore it is useful to describe HA gel formation in presented fashion. Due to the nature of HA:PL interactions polymer dynamics description approaches such as Rouse model are not useful.

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## References

- [1] Dédinaté, A.: Biomimetic lubrication. *Soft Matter*, **8**, 273-284 (2012)
- [2] M. Doi, S. F. Edwards, *The theory of polymer dynamics*, (Oxford University Press, Oxford, 1994)
- [3] Gadomski, A.: Nucleation-and-growth problem in model lipid membranes undergoing subgel phase transitions is a problem of time scale. *Eur. Phys. J. B*, **9**, 569-571 (1999)
- [4] Goudoulas, T.B., Kastrinakis, E.G., Nychas, S.G., Papazoglou, L.G., Kazakos, G.M., Kosmas, P.V.: Rheological study of synovial fluid obtained from dogs: healthy, pathological, and post-surgery, after spontaneous rupture of cranial cruciate ligament. *Ann. Biomed. Eng.*, **38**, 57-65 (2010)
- [5] G.G. Hari, C.A. Hales, *Chemistry and Biology of Hyaluronan*, Elsevier Science, Amsterdam, (2008)
- [6] Haubol, H.J., Matha, A.M., Saxena, R.K.: Mittag-Leffler functions and their applications. *Journal of Applied Mathematics*, **2011**, (2011)
- [7] Hills, B.A.: Boundary lubrication in vivo. *Proc. Inst. Mech. Eng. H*, **214**, 83-94 (2000)
- [8] Jung, S., Petelska, A., Bełdowski, P., Augé, W., Casey, T., Walczak, D., Lemke, K., Gadomski, A.: Hyaluronic acid and phospholipid interactions useful for repaired articular cartilage surfaces - a mini review toward tribological surgical adjuvants. *Colloid Polym. Sci.*, **295**, 403-412 (2017)
- [9] Nitzan, D.W., Nitzan, U., Dan, P., Yedgar, S.: The role of hyaluronic acid in protecting surface?active phospholipids from lysis by exogenous phospholipase A2. *Rheumatology*, **40**, 335-340 (2001)
- [10] Panja, D.: Anomalous Polymer Dynamics Is Non-Markovian: Memory Effects and The Generalized Langevin Equation Formulation. *J. Stat. Mech.*, **6**, P06011 (2010)
- [11] Pasquali-Ronchetti, I., Quaglino, D., Mori, G., Bacchelli, B., Ghosh, P.: Hyaluronan-phospholipid interactions *J. Struct. Biol.*, **120**, 1-10 (1997)
- [12] Podlubny, I., *Fractional differential equations*, (Academic Press, San Diego, 1999)
- [13] Podlubny, I.: Matrix approach to discrete fractional calculus. *Fractional Calculus and Applied Analysis*, **3**, 359-386 (2000)

- [14] Richmond, T.J.: Solvent accessible surface area and excluded volume in proteins. Analytical equations for overlapping spheres and implications for the hydrophobic effect. *J. Mol. Biol.*, **178**, 63-89 (1984)
- [15] Rouse, P. E.: A Theory of the Linear Viscoelastic Properties of Dilute Solutions of Coiling Polymers. *J. Chem. Phys.*, **21**, 1272-1280 (1953)
- [16] Samko, S. G., Kilbas, A. A., Marichev, O. I.: *Fractional integrals and derivatives - theory and applications*, (Gordon and Breach, Amsterdam, 1993)
- [17] Siódmiak, J., Beldowski, P., Augé, W., Ledziński, D., Śmigiel, S., Gadomski, A.: Molecular Dynamic Analysis of Hyaluronic Acid and Phospholipid Interaction in Tribological Surgical Adjuvant Design for Osteoarthritis. *Molecules*, **22**, 1436-1456 (2017)
- [18] Sorkin, R., Kampf, N., Zhu, L., Klein, J.: Hydration lubrication and shear-induced self-healing of lipid bilayer boundary lubricants in phosphatidylcholine dispersions. *Soft Matter*, **12**, 2773-2784 (2016)
- [19] Wang, M., Liu, C., Thormann, E., Dédinaité, A.: Hyaluronan and phospholipid association in biolubrication. *Biomacromolecules*, **14**, 4198-4206 (2013)
- [20] Wieland, D.C.F., Degen, P., Zander, T., Gayer, S., Raj, A., An, J., Dédinaité, A., Claesson, P., Willumeit-Ramer, R.: Structure of DPPC-hyaluronan interfacial layers? effects of molecular weight and ion composition. *Rheumatology*, **412**, 729-740 (2016)
- [21] yasara, <http://yasara.org/>