

## **Phospholipids self-assembling in the presence of ionic microgels and hydrophobically modified polyelectrolytes to obtain biomimetic lubricants**

**Acronym: TriboGelPEL**  
**Contract no: 92 BM / 2017**

**Romanian partner: "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania**  
**Foreign partner: National Institute of Applied Sciences, Lyon, France**

### **Context:**

The importance of obtaining new lubricants lies in the large number of patients suffering from osteoarthritis which required prosthetics as unique treatment. It is known that osteoarthritis is installed mostly as a result of aging, but can have other causes such as trauma, infection, prolonged exercise or obesity. In all cases it is about a wear of articular cartilage, wear that can lead to a severe suffering if no treatment is established. Despite many efforts to develop new wear-resistant materials for joint implants, their lifetime in vivo is often much shorter than the ones extrapolated from ex vivo simulations. This discordance is mainly due to the simulation conditions. One of the conditions is that the lubricants used do not reproduce entirely the mechanical and physico-chemical properties of the biological lubricant.

In this respect, the main purpose of the project was to obtain a biomimetic model of synovial fluid with composition and molecular assemblies similar to those from the natural lubricant, that could be used as lubricant for testing the lifetime of the osteoarticular implants.

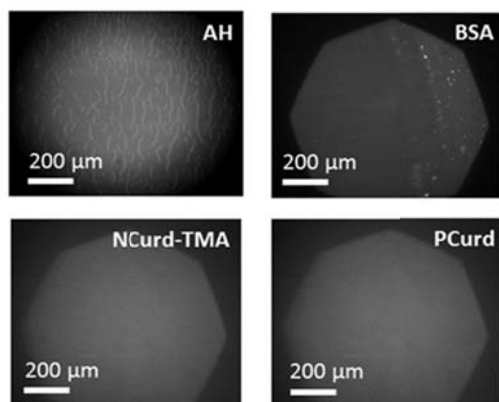
### **The main objectives of the project were:**

#### **E1. Obtaining and characterization of ionic microgels from natural and synthetic PELs**

Polyelectrolytes (PEL) as polysaccharide type (curdlan, hyaluronic acid) with anionic (phosphate) and cationic (quaternary ammonium) groups were synthesized. The water-soluble synthesized derivatives were purified by diafiltration and recovered by lyophilization and characterized by FTIR and NMR spectroscopy. The degree of substitution (0.65 for PCurd, 1 for PHA, 0.15 NCurd-TMA, 0.08 NCurd-TEA) was calculated from information obtained from both NMR spectroscopy and conductometric titrations.

Synthesized polysaccharide derivatives could be used as a component of biomimetic lubricants that could mimic the physico-chemical and tribological properties of synovial fluid. In this context, additional studies have been conducted by interaction with proteins (a synovial fluid component), taking bovine serum albumin (BSA) as a model.

An important goal of the project was to test the lubricity properties of the obtained compounds. Using a homemade tribometer (developed by the French partner, LaMCoS), the friction coefficient between a smooth glass surface and a HEMA elastic convex lens was measured, each surface being pre-coated with a fluorescently marked lipid bilayer (phospholipids, PL). To measure the friction coefficient, a layer of lubricant (compounds synthesized by the Romanian partner, LPNMBB) was interposed between the two support layers and the friction tests were performed for one hour. Visualization of the contact was done using a confocal microscope after the friction test (Figure 1).



## **E2. Obtaining and physico-chemical characterization of self-assembled PL vesicles in the presence of ionic microgels**

PL vesicles were obtained by hydrating a thin layer of phospholipids (Emulmetik 900). The obtained suspension was sonicated and then extruded through a membrane with pore size of 0.45  $\mu\text{m}$ , when the solution becomes almost transparent. DLS measurements confirmed the obtaining of vesicles with a size of 100 nm. In preformed vesicle solution, previously obtained ionic microgels were introduced and incubated for several hours. EDX spectra have shown phospholipid assembling on ionic microgels by the presence of phosphorus on their surface.

To demonstrate the coating of lipid-coated microgels, the microgels were previously loaded with a dye (Rhodamine 6G). Dye release has been shown to be faster in uncoated microgels than in those coated with lipid layers, demonstrating the assembly of phospholipids on their surface that prevents the release of the dye.

## **E3. Obtaining and characterization of natural and synthetic PEL hydrophobic modified**

The MA-St alternating copolymer was hydrophobic modified by amidation reaction with alkylamines. Thus, derivatives with octylamine and dodecylamine with varying degrees of substitution (between 84 and 10%) were obtained. They were hydrolyzed and then purified by advanced diafiltration and lyophilization.

The structure of the derivatives was confirmed by FTIR spectroscopy, and the degree of substitution of the hydrophobic polymers was determined by conductometry and from  $^1\text{H}$  NMR spectra. The derivatives were also characterized by potentiometric titration in excess of base or acid. In the pH curve at the titration of the derivative, one or two equivalence points corresponding to the free carboxylic groups remaining after the removal of the maleic acid but also of the adjacent carboxylic groups, resulting from the hydrolysis of the unmodified anhydride cycles could be observed. Unlike the starting copolymer, poly (maleic acid-alt-styrene), the obtained amphiphilic derivatives are soluble only at basic pH, the pH at which the solubilization occurs depending on the degree of substitution and the length of the hydrophobic chain introduced.

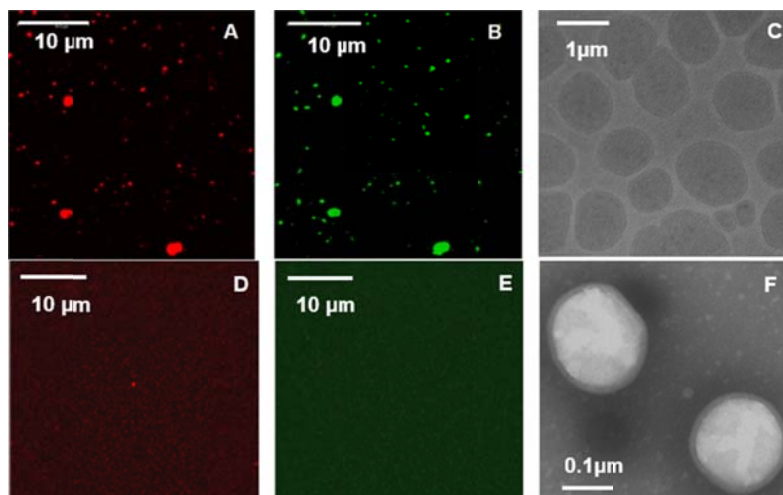
Aggregation of hydrophobic modified polyelectrolytes was studied by fluorescence using the pyrene as probe, the ratio of the intensity of the third and first emitted peak spectra ( $I_3 / I_1$ ) being a measure of polarity in microheterogeneous systems. Thus, at very low concentrations of the derivatives, the ratio  $I_3 / I_1$  is close to that of the water (0.6), but with the increase of the concentration of the polymer, it starts to accumulate and the pyrene molecules are solubilized in the hydrophobic microdomains formed, the ratio  $I_3 / I_1$  increasing. The critical aggregation concentration decreases from 0.25 g/L of the poly (maleic-alt-styrene) copolymer at 0.01 g/L for the octyl amine derivative and a 12% substitution degree to 0.0001 g/dL to the derivative with a degree of substitution of 78%.

The results concerning the synthesis and characterization of hydrophobic modified polyelectrolytes were presented at the Days of "Alexandru Ioan Cuza" University of Iasi, October 25-26, 2018 („Hydrophobic modified poly(maleic anhydride-alt-styrene) for preparation of nanosized particles”, authors I.M. Pelin, D.M. Suflet, I. Popescu).

## **E.4. Obtaining and physico-chemical characterization of self-assembled PL vesicles in the presence of hydrophobic modified PEL**

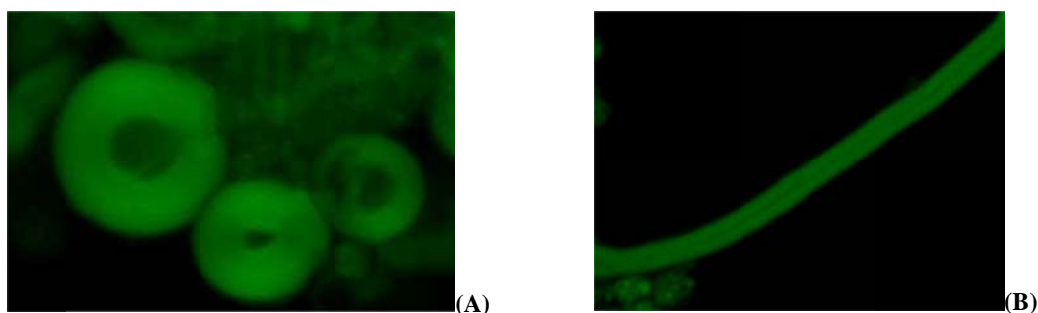
Two methods of self-assembly of PL in the presence of polysaccharides were used to reproduce a healthy biomimetic synovial fluid composition: 3 mg / ml polysaccharide, 0.2 mg / ml lipids, 20 mg / ml serum proteins. Using two different fluorescents (green for the polysaccharide component and red for the lipid component) it was shown that the second manufacturing method allows for a concentrated suspension of micrometric vesicles

comprising the whole polysaccharide component. This biomimetic solution was called "gel in", unlike the first manufacturing method that allows for a homogeneous solution in which the lipid component remains inside the polysaccharide component, when biomimetic solution was named "gel out" (Figure 3).



**Figure 3.** A, B, D, E represents CLSM images: the vesicles present both fluorescentes, overlaped in the case of biomimetic liquid "gel in" (A, B) and a uniform fluorescence on the hole image in the case of biomimetic liquid "gel out" (D,E). C and F are TEM images of vesicles with a diameter of 2μm for biomimetic liquid "gel in" and of about 200 nm for biomimetic liquid "gel out"

For "gel in" solutions, a difference was observed between macromolecules of phosphorylated hyaluronic acid forming spherical vesicles, while the phosphorylated curdlan macromolecules form rather cylindrical structures (Figure 4).



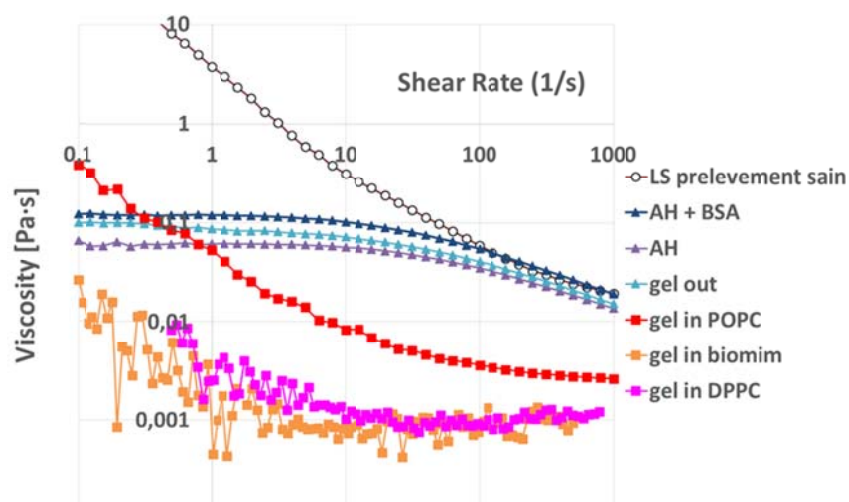
**Figure 4.** Spherical vesicles of phosphorylated hyaluronic acid (A) and cylindrical structures of phosphorylated curdlan (B)

#### **E.5. Tribological characterization of self-assembled PL vesicles in the presence of ionic microgel and PEL hydrophobic modified**

To test the mechanical / tribological properties of the two types of PL / PEL, "gel in" structures compared to "gel out", a biotribometer performed by the LaMCoS team was used, in the ex vivo model configuration of articular contact. Three types of biomimetic lubricants were used on the knee simulator and two main types of in vivo "wear mechanisms" were

highlighted: 1) a mechanism showing a high wear rate with small wear and tear particles material - *abrasive wear*; 2) a mechanism showing a wear rate ten times lower than in the previous case, with large particles - *adhesive wear*. These two types of wear are considered to be related to the biological environment (synovial fluid) with an inflammatory nature in the first case and non-inflammatory in the second case.

From the rheological studies, it is pointed out that the "gel out" curves appear to have the same look with the curve of the harvested synovial fluid, but the viscosity values are different. At a shear rate of  $2.5 \text{ s}^{-1}$ , which corresponds to the physiological velocity, it was found that the viscosity of the harvested synovial fluid sample is 100 times higher than the biomimetic fluid "gel in POPC" and 10000 times higher than the biomimetic fluid "gel in DPPC" or "gel in biomimetic" (Figure 5).



**Figure 5.** Rheological results of different biomimetic solutions

The biomimetic solutions obtained in the laboratory have not yet entirely succeeded in imitating the rheological behavior of the harvested synovial fluid, probably an important component was not reproduced. It has been noticed that lipid layers have degraded in FRET (Fluorescence Resonance Energy Transfer) analysis, and for this reason another method, Reflection Interference Contrast Microscopy (RICM) has been chosen, whereby the thickness of the lubricated film was inhomogeneous and ranging from a few hundreds of nanometers to a few micrometers.

Some of the obtained results were disseminated by the French partner at the 45th Tribology Symposium Leeds-Lyon, September 4-7, 2018, Leeds, England, with the paper: "Role of polysaccharides – phospholipids interactions in the tribological behavior of synovial fluid substitutes", authors: Mirela-Maria Sava, Nesrine Daragi, Frederic Hab, Dana M. Suflet, Irina Popescu, Irina M. Pelin, Bernard Tinland, Ana-Maria Trunfio-Sfarghiu.

Additional experiments are required to obtain biomimetic fluids with optimal properties as to be used as substitutes for synovial fluid and to meet the standards for use in joint prosthesis simulators.