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20th IUPAB Congress
45th Annual Meeting of SBBf, and 50th Annual Meeting of SBBq
October 4th to 8th, 2021 – Virtual Meeting

A biomimetic device combining microfluidics with nanotechnology allows studying the adhesion of erythrocytes to blood vessels.

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Erythrocytes under pathological conditions undergo eryptosis, a process characterized by biochemical and morphological changes such as phosphatidylserine (PS) exposure to the plasma membrane external layer. Eryptotic erythrocytes adhere to the endothelial cells (ECs), which may be important in the pathology of bacterial infections and congenital diseases like sickle cell disease. Externalized PS can bind to receptors expressed on ECs under pathological conditions. To understand the adhesion mechanism, we designed a device combining microfluidics with nanostructured surfaces to mimic the capillary architecture and the expression of adhesive molecules by the activated ECs.

Microfluidic chips were prepared in PDMS using molds fabricated by photolithography. Nanostructured surfaces were synthesized by block copolymer lithography and consisted of a glass surface covered with 7 nm diameter gold nanoparticles (AuNPs), arranged in a quasi-hexagonal array. The microfluidic chip was adhered to the nanostructured surface by an O_2 plasma treatment. The AuNPs were functionalized with a polyethylene glycol chain (PEG) that binds to the AuNPs by a thiol at one end and has a nitriloacetic group (NTA) at the other end. The NTA binds proteins expressing a His-Tag. The surface not occupied by AuNPs was covered with PLL-g-PEG.

We corroborated the specific AuNPs functionalization by quartz microbalance and fluorescence microscopy using a GFP-His Tag. Then, we studied the erythrocytes adhesion to a device functionalized with Annexin V-His Tag at different flows. Two conditions that promote eryptosis, incubation at 50° C or treatment with ionomycin, significantly increased the adhesion of erythrocytes at flows up to 1.5 dyn/cm^2, in comparison with untreated erythrocytes. However, eryptotic erythrocytes also showed adherence to a device functionalized with a PEG lacking NTA groups. This indicates that erythrocytes adhesion may not be mediated only by PS receptors.

Future experiments will test the erythrocytes adhesion elicited by proteins usually expressed by the activated ECs.

Keywords: biomimetic, phosphatidylserine, endothelium
Funding: FONCYT (PICT-2019 03218), CONICET, The Company of Biologists (travel grant), UBACYT 20820160401330BA.