SAB 2020

Biofísica en tiempos de COVID-19

Libro de Resúmenes

3 y 4 de diciembre de 2020
Argentina

Sociedad Argentina de Biofísica
Biofísica en tiempos de COVID-19 : Primeras Jornadas Virtuales SAB 2020 / compilado por José M. Delfino ... [et al.]. - 1a ed. - Buenos Aires : SAB - Sociedad Argentina de Biofísica, 2020.
Libro digital, PDF

Archivo Digital: descarga y online
ISBN 978-987-27591-8-6

1. Biofísica. 2. Investigación Experimental. I. Delfino, José M., comp. I. Título.
CDD 571.4

Diagramación y Edición

Ernesto Ambroggio, Soledad Celej, Axel Hollmann, Juan Pablo Acierno

Diseño de Tapa y Logo

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Juan Pablo Acierno

Sociedad Argentina de Biofísica

Member of the International Union for Pure and Applied Biophysics

Primeras jornadas virtuales de la Sociedad Argentina de Biofísica

3 y 4 de diciembre 2020

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A biomimetic approach to study human erythrocytes adhesion to vascular endothelium induced by alpha-hemolysin.

Saffioti NA[a], Leal Denis MF[b], Álvarez C[b], Herlax V[c], Schwarzbaum P[b], Pallarola D[a]
a - Instituto de Nanosistemas, Universidad Nacional de General San Martín, Av. 25 de Mayo y Francia, San Martín, 1650, Argentina
b - CONICET – Universidad de Buenos Aires. Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini" (IQUIFIB). Buenos Aires, Argentina.
c - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

The α-haemolysin (HlyA) is an exotoxin produced by several strains of uropathogenic *E. coli*, one of the most important etiological agents of urinary infections. HlyA irreversibly binds to human erythrocytes (RBCs), initiating a degenerative process called eryptosis, characterized by biochemical and morphological changes such as phosphatidylserine (PS) exposure to the external layer of the plasma membrane of RBCs, shrinkage, and swelling. HlyA-induced PS externalization can lead to adhesion of RBCs to vascular endothelial cells (VECs).

We studied the capacity of HlyA-treated RBCs to adhere to: 1- activated endothelial HMEC-1 cells under different flow conditions (dynamic adhesion); 2- surfaces homogeneously covered with extracellular matrix components in static conditions.

Results showed that HlyA induced adhesion of RBCs to VECs at low flow (0.2 dyn/cm^2), although higher flows induced rapid detachment. On the other hand, HlyA treatment also induced static adhesion of RBCs to collagen or fibrinogen. Thus, HlyA-treated RBCs displayed high but weak adherence to VECs under the experimental conditions.

Additionally, to study the molecular mechanism of the HlyA-induced adhesion of RBCs we designed a biomimetic device to emulate the conditions of the blood vessels.

The device was built by coupling a microfluidic chip to a nanopatterned surface (NPS) coated with gold nanoparticles (AuNPs). Different adhesion molecules from the VECs could be anchored to the AuNPs to mimic exposure of adhesion molecules of an activated endothelium whereas, the architecture of the capillaries was emulated by a network of microfluidic channels built in polydimethylsiloxane (PDMS). The synthesis of the device was optimized by following the process with quartz microbalance and fluorescence microscopy.

Future experiments using the device will allow investigating HlyA-induced adhesion of RBCs to specific adhesion molecules under flow conditions.

Acknowldegments

The author acknowledges a travel grant for The Company of Biologist that made possible this work.

*Both authors contributed equally