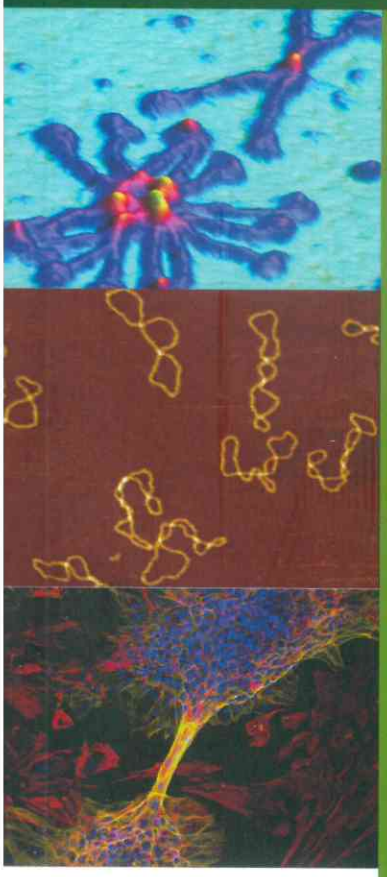
Volume 48 • Supplement 1 • July 2019

European Biophysics Journal

with Biophysics Letters



JOINT 12th EBSA congress and 10th ICBP – IUPAP congress
July 20–24, 2019, Madrid, Spain

Programme Book and Abstracts

Published on behalf of the
European Biophysics Societies' Association

Springer

EBSA
Biophysics in Europe

P-082

Shear flow promotes isotropic redistribution of fibrin fibers inside glycosylated fibrin networks

I. Piechocka[1], N. Wolska[2], B. Luzak[2].
[1]Institute of Fundamental Technological Research Polish Academy of Sciences (IPPT PAN), Warsaw, Poland; [2]Medical University of Lódź, Lódź, Poland.

Fibrin networks form at the border of injured blood vessel wall, preventing bleeding and promoting wound repair. *In vivo,* the formation of fibrin clots takes place in the presence of flowing blood that exerts a continuous shear force on the structure. While it is commonly known that the external force affects mechanical properties of fibrin clots, the exact role of shear flow in the bulk organization of fibrin networks and the response of individual fibrin fibers within fibrin clots still remains poorly understood. Here, by using combined mechanical (parallel-plate flow chamber) and optical (confocal microscopy) methods we follow *in situ* changes in the spatial organization of individual glycosylated fibrin filaments in the presence of shear flow. Glycation of fibrinogen, and subsequently fibrin, is a natural process taking place under normal physiological conditions. However, excess glycation, as observed in diabetes states, yield formation of modified fibrin clots with more compact and difficult to lyse structure. We show that shear flow deformations can directly affect orientation of individual glycosylated fibrin fibers by promoting their highly isotropic distribution within fibrin clots. Such shear flow-induced reorientation of fibrin fibers may impact the bulk permeability of the whole clot, and consequently influence the diffusion of blood cells and/or thrombolytic components through fibrin mesh. Our results highlight thus the direct role of shear flow in reorganization of fibrin networks at the level of individual fibrin filaments, and reveal its importance in modulating the response of fibrin clots in diabetic conditions.

P-083

Supracellular Mechanical Architecture of the Intact Bone Microenvironment

X. Chen, R. Hughes, N. Mullin, R. Hawkins, I. Holen, N. Brown, J. Hobbs.
University of Sheffield, Sheffield, United Kingdom.

We introduce an improved platform based on atomic force microscopy (AFM) to quantify the mechanical architecture of the intact bone microenvironment (BMev) within regions of interests (metaphysis, cortical bone, marrow and growth plate). The elastic modulus of all regions was found to be highly heterogeneous on supracellular scale, ranging over 3 to 4 magnitudes, and could be down to only few Pas. Such unique architecture provides 'soft channels' for cell migration and consequently may impact a substantial number of active biological processes involving cell migration, such as bone remodelling and cancer metastasis.

We collected the force generated by indentation of the fresh (un-fixed) murine bone tissue in physiological buffer and the subsequent relaxation process, on either individual points distributed over the bone surface or a micro scale array (*i.e.* AFM force map). The Young's moduli, extracted from weighted Sneddon model fit, of all BMev regions are overall much lower than the values from individual cells or bulk tissues. This is likely due to the tissue hydration and natural supracellular structures maintained relatively intact in our study. The viscoelastic model describes the mechanical response better than elastic model, and indicates that the instantaneous elastic response can be neglected in almost all BMev regions. Both elastic and viscoelastic properties were found to be significantly different between the various BMev regions as well as between bones from young and mature mice.

High resolution AFM force maps show highly heterogeneous mechanical properties and corresponding morphology at a supracellular level, in particular in the metaphysis and cortical bone. We also demonstrate the ability to further correlate such maps with tissue components by combining *in-situ* and *ex-situ* fluorescent BMev images. This improved AFM based system is powerful for further characterisations of bones in the presence of stimuli (*e.g.* hormones, cancer cells and drugs) or other complex tissues.

P-084

Super-resolution microscopy reveals nano-hubs of spatially segregated proteins within focal adhesions

S. Keary, N. Mateos, F. Campelo, M. Garcia-Parajo.
ICFO- The Institute of Photonic Sciences, Castelldefels, Spain.

Focal Adhesions (FA) are mechanosensitive complexes that connect the extracellular matrix (ECM) with the actin cytoskeleton. This is achieved through clustered integrins, their ligands in the ECM, and an internal dynamic protein complex linking the cytosolic domain of integrins with the actin cytoskeleton. In contrast to the established view that FAs are homogenous micron-scale protein assemblies, recent super-resolution imaging and single molecule dynamic approaches are challenging this view. These studies suggest that FA molecular components are highly organized in the axial direction establishing segregated layers of functional activity. Recent data also indicates that similar type of nanoscale modularity might exist in the horizontal plane of FAs. Here, we present a set of experiments aimed at dissecting the lateral nanoscale organisation of different proteins as a function of FA maturation. By combining quantitative multicolour STED and STORM nano-scopy we revealed that main protein actors involved in FAs, i.e. paxillin, talin and vinculin, are organised in segregated nanoclusters within FAs. Whereas paxillin nanoclusters are mostly concentrated inside FAs, both vinculin and talin nanoclusters were found inside and outside mature FAs, albeit at different densities and molecular packing. Paxillin and vinculin nanoclusters inside FAs are spatially segregated at around 80nm while talin nanoclusters were found to be more sparsely distributed and exhibit a larger variation in nanocluster sizes and molecular density. This larger heterogeneity of talin organization might result from the highly dynamic spatiotemporal organization and segregation of inactive *vs.* active integrin nanoclusters within FAs. As a whole, our data indicate a highly complex spatiotemporal organization within FAs with different proteins forming nano-hubs of activity. The duration and strength of the protein interactions inside these hubs are highly regulated not only in the axial direction but, importantly, also in the horizontal plane of FAs.

P-085

Bacterial cell wall mechanical damage studied by simultaneous nanoindentation and fluorescence microscopy

A. Del Valle[1], P. Bondia[1], C.M. Tone[1], V. Vadillo[2], C. Flors[1].
[1]IMDEA Nanoscience, Madrid, Spain; [2]Department of Applied Physics (University of Extremadura), Badajoz, Spain.

We have developed an experimental protocol to perform simultaneous AFM nanoindentation and fluorescence imaging on immobilized bacterial cells, with the goal of finding potential correlations between nanoindentation conditions and damage to the bacterial cell wall. The latter is assessed by quantifying the fluorescence enhancement kinetics arising from propidium iodide (PI), a marker for membrane integrity. Two types of bacteria with different cell wall composition, *E. coli* (Gram-negative) and *B. subtilis* (Gram-positive) are compared. Our main observation is that a correlation exists between the magnitude of the force applied to rupture the cell wall and the delay of the PI fluorescence response. We have also observed that there are other influential parameters in these experiments, such as the bacterial immobilization method or the cell cycle phase. Indeed, bacteria are easier to puncture during or just after replication. Other parameters, such as tip geometry or indentation pattern, are being investigated. While previous studies have shown that bacteria are rather resilient to AFM nanoindentation [1], our experimental strategy using simultaneous fluorescence detection of PI in a systematic and quantitative way may help to provide a deeper insight into the range of forces that are relevant to "mechano-bactericidal" mechanisms of action [2].

[1] Z. Suo, R. Avci, M. Deliorman, X. Yang, and D. W. Pascual, "Bacteria survive multiple puncturings of their cell walls," *Langmuir*, vol. 25, no. 8, pp. 4588–4594, 2009.
[2] C. Moraes, R. D. Rogers, P. Berton, N. Lin, and N. Tufenkji, "Nanodarts, nanoblades, and nanospikes: Mechano-bactericidal nanostructures and where to find them," *Adv. Colloid Interface Sci.*, vol. 252, pp. 55–68, 2017.