THE ROLE OF MEMBRANE LIPID RAFTS IN OSTEOBLASTIC SENSING AND PROPAGATION OF MECHANICAL FORCES: A MICROFLUIDIC-BASED SINGLE CELL ANALYSIS STUDY

Bibhas Roy[1†], Dario Carugo[3†], Xunli Zhang[3], Tapas K. Maiti[1] & Suman Chakraborty[2*]

[1]Department of Biotechnology, Indian Institute of Technology, Kharagpur, India

[2]Department of Mechanical Engineering, Indian Institute of Technology, Kharagpur, India

[3]Bioengineering Sciences Group, Faculty of Engineering and the Environment, University of Southampton, UK

[†]Authors have equally contributed to the work

ABSTRACT:

With the advent of microfluidic devices it has become possible to control on-demand the properties of fluidic environments at the microscale. This has opened the way to the development of microfluidic systems capable of analysing the response of individual cells under exposure to a range of different physical and chemical stimuli. This methodology, which has been defined by many as single-cell analysis (SCA), has permitted to gain a deeper understanding of the mechanisms with which cells transduce extracellular stimuli into intracellular biophysical and biochemical activities. However, it still remains unclear how this transduction process develops in osteoblast cells and which are the key governing parameters. In the present study we report a microfluidic-based SCA technique for investigating the mechanisms of osteoblast mechanotransduction under exposure to fluid shear stress (FSS). With the developed method we quantified local cellular adaptations under exposure to FSS, and we found that membrane cholesterol-rich domains (lipid rafts, LRs) acted as one of the main regulators of cellular adaptation. LRs content was found to be correlated with the extent of cell membrane deformation, stress fiber formation and change of traction force at the increased number of focal adhesion points (FAPs).

KEYWORDS:

Mechanotransduction; Membrane deformation; Lipid rafts; Force adaptation; Stress fibers; Cellular traction force

INTRODUCTION:

Cellular mechanotransduction is the functional translation of external physical stimuli into intracellular biophysical and biochemical responses. Despite the governing mechanisms have been widely investigated, the process of cell adaptation under mechanical stress remains grossly elusive mainly due to the lack of biomimetic experimental conditions capable of reproducing the physiological circumstances. With the advent of microfluidics, a range of mechanical stimuli can be applied on individual cells, and cell response can be monitored *in situ* through SCA [1, 2]. SCA studies suggested that FSS-induced cellular response may be modulated by the spatial heterogeneity of cell membrane structure and the dynamics of membrane physical properties, including the content of LRs. It has been previously observed that membrane physical properties, such as fluidity and viscoelasticity, are governed by the internalization and externalization of LRs between cell membrane and vesicle membranes. However, the dynamic behaviour of LRs under biomimetic fluidic conditions and how the interaction between LRs and other cellular mechanosensors (i.e. membrane receptors, ion-channels and focal adhesions) affects the propagation of mechanical forces remain widely overlooked, and represent the subject of the present investigation.

EXPERIMENTAL:

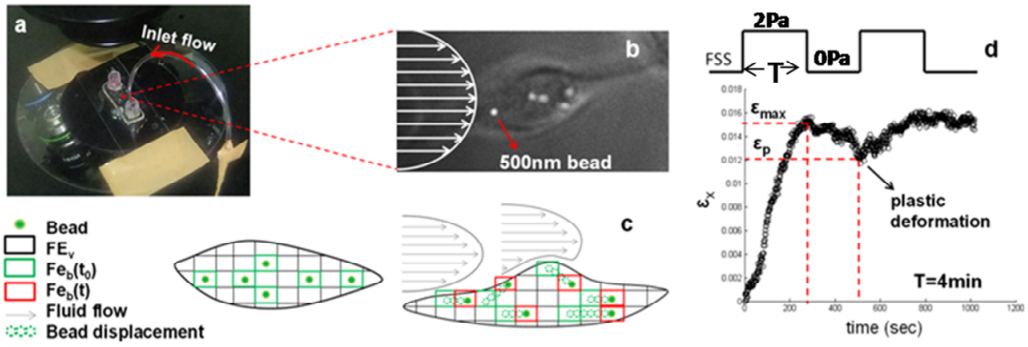

Figure 1 – (a) Hybrid PDMS-glass microfluidic device. (b) Microscope image of single MG63 osteoblast cell with membrane-linked 500nm diameter fluorescent beads. (c) Schematic depiction of the analysis system. Beads displacement is tracked for calculating cell membrane deformation, and a finite element (FE) analysis is performed to calculate LR content. (d) Representative membrane deformation (ε_x) trend, with identification of maximum deformation (ε_{max}) and plastic deformation (ε_p). FSS pulse duration, T = 4min.

Human osteoblasts are exposed to different mechanical stimuli, including FSS generated by the fluid flow within the lacunar and canalicular networks of bone during the cyclic excercise. In the present study, human osteoblast cell line, MG63, was used as a model mechanoresponsive cell. A novel microfluidic-based method was developed in order to investigate the relationship between membrane deformation and the spatio-temporal dynamics of LRs under exposure to both steady and unsteady (i.e. pulsed) FSS. Briefly, 500nm diameter fibronectin-coated fluorescent beads were attached to the MG63 cell membrane and cells were seeded within hybrid PDMS-glass microfluidic channels (Fig. 1a-b).

FSS, either steady or unsteady, was generated by pumping 1% serum containing media through the microfluidic channel, using programmable syringe pump and solenoid valve. In order to quantify membrane deformation dynamics under varying FSS levels, membrane-linked beads were simultaneously tracked by using confocal laser scanning microscopy and a MATLAB-based multiple-particle tracking algorithm (Fig. 1c-d). Membrane fluidity and LRs dynamics were quantified by fluorescent labelling, using 4-(Dicyanovinyl) Julolidine (DCVJ) molecular rotor and cholera toxin subunit B(CTxB) conjugated with alexafluor-594, respectively. The mechanisms of FSS sensing and FSS-induced intracellular biochemical adaptations were also elucidated by probing the sub-cellular change in actin polymerization, by GFP-Actin transfection, and cell traction force, by using an in-house traction force microscopy technique [3].

RESULTS AND DISCUSSION:

Results showed that the dynamics of cell membrane deformation was mainly regulated by the dynamics of the applied FSS stimulus, and two main phases of cell membrane deformation could be detected. With immediate onset of the flow, membrane deformed predominantly in the flow direction (i.e. stress-governed deformational phase). When the flow was stopped, membrane tended to recover its initial configuration (i.e. recovery phase). A residual plastic deformation was detected at the end of the recovery phase, which was observed to depend on the duration of the FSS pulse (Fig. 2c) and LRs content. Interestingly, a correlation between local LRs content and membrane deformation was observed (Fig. 2d). During stress-governed deformational phase, the overall LRs content was observed to increase. Conversely, membrane content of LRs was observed to decrease during the recovery phase. This observation clearly shows that cells adapt to physical force exerted in the form of FSS, which cause cell membrane to deform or potentially being damaged, with an increase of LRs content. Such increase is likely associated with increased membrane rigidity and reduced membrane recovery time.

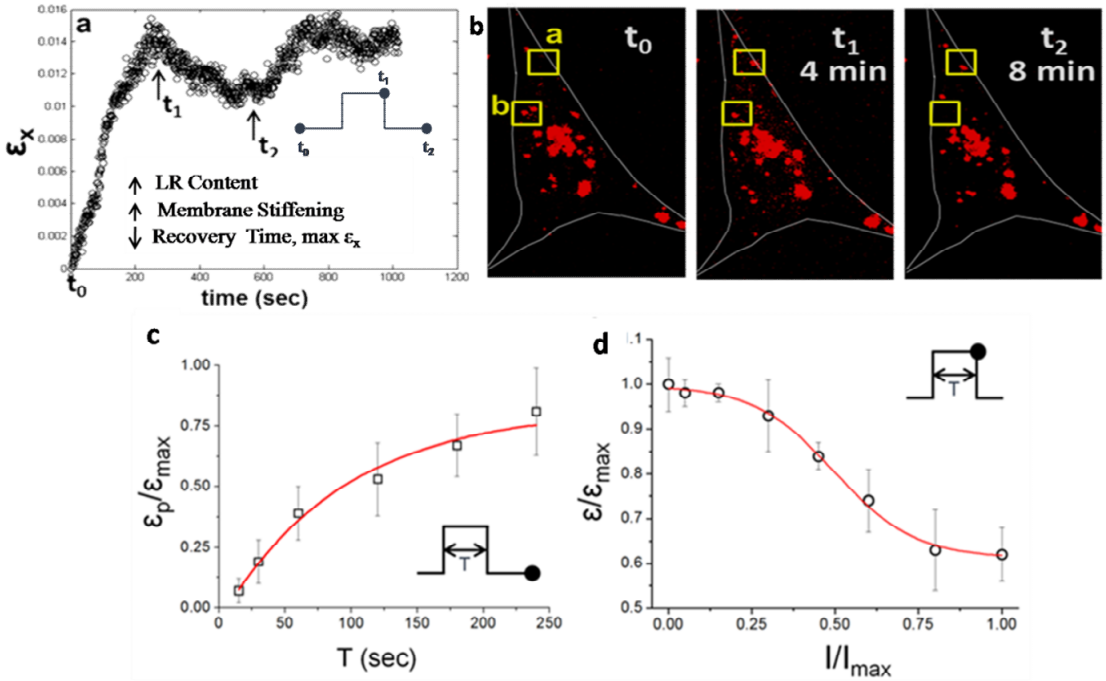

Figure 2 – (a) Representative membrane deformation (ε_x) under exposure to pulsed FSS. (b) Fluorescent images of CTxB-Alexafluor 594 labelled LRs, indicating LRs content and their localization in the membrane at different timescale during pulsed FSS. (c) Dependence of plastic deformation, ε_p, on the duration of the FSS pulse (T). (d) Correlation between local deformation and lipid raft content (I). T = 4min. Deformation and LRs content are normalised with respect to the maximum value.

For better adaptation under FSS, the internal force propagation was initiated at the membrane LRs-rich region, *via* increased actin polymerization near the membrane (Fig. 3). The polymerized actin-network helped MG63 to interact more

strongly with the substrate in order to prevent detachment by formation of more focal adhesion points (FAPs). In this adaptation process, the increased amount of LRs mediated by applied physical forces initiated the process of actin polymerization and stress fiber formation by activation of Rho-GTPase and formation of Arp2/3 complexes. The strength of cellular skeletal backbone was increased by actin polymerization process and the cell attachment strength to the substrate was increased by stress fiber formation at FAPs. The latter phenomenon resulted in initial increase of cell traction force near the membrane region; which however was observed to decrease at long-term exposure to FSS (Fig. 4). Osteoblastic force sensing and propagation were significantly decreased by treatment with methyl-β-cyclodextrin and increased by cholesterol (results not shown), demonstrating the role played by LRs in the mechanotransduction process.

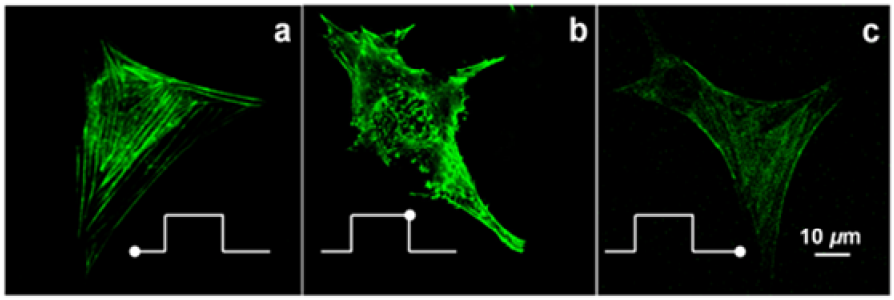

Figure 3 -GFP-actin dynamics during shear stress adaptation process. (a) At $t=t_0$ actin network of MG63 was long expanded and a reduced number of FAPs was detected. (b) After application of FSS, at $t=t_1$, actin network was more branched and an increased number of FAPs was detected, which were localized mainly at the membrane region. (c) Subsequently, cell tended to regain its primary actin structure after flow was stopped.

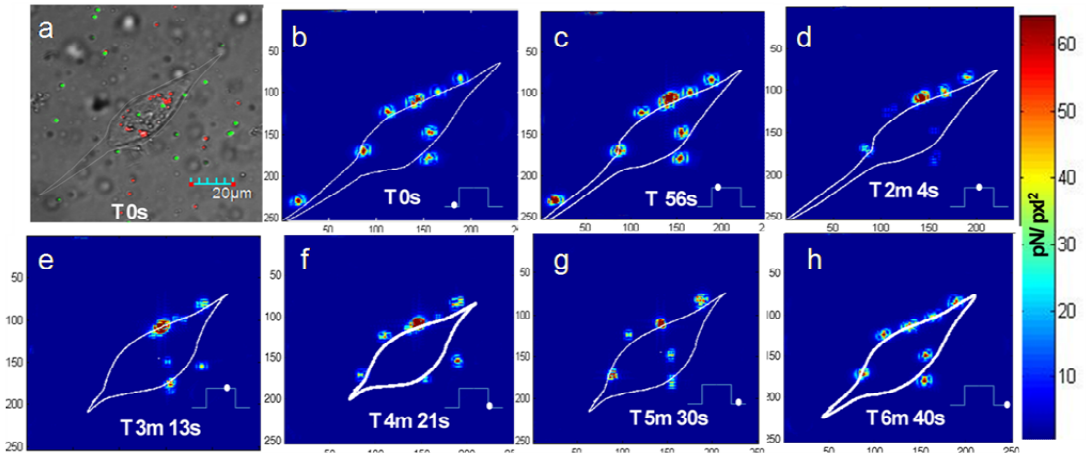

Figure 4– Quantification of traction forces exerted on the substrate by single cell subjected to pulsed FSS. (a) Merged picture of cell (bright field), fibronectin coated beads (red fluorescent) attached to the membrane, and polystyrene beads (green fluorescent) embedded within soft PDMS substrate. (b-e) Traction force field at different time intervals during application of pulsed FSS. T=3 sec; 1pixel=0.482μm.

CONCLUSION:

In the force-mediated adaptation of MG63, LRs play major role by initiation of the biochemical signalling, including increase of actin stress fibers and traction force at FAPs. Hence, it can be concluded that osteoblast mechanotransduction may be predominantly regulated by LRs, and unique dynamic couplings between membrane deformation, LRs content and intracellular signals occur during a mechanical sensing process.

REFERENCES:

[1] D. Di Carlo and L. P. Lee, *Analytical Chemistry*, 2006, **78**, 7918-7925.
[2] D. Lingwood, H. J. Kaiser, I. Levental and K. Simons, *Biochemical Society Transactions*, 2009, **37**, 955-960.
[3] T. Das, T. K. Maiti and S. Chakraborty, *Lab Chip*, 2008, **8**, 1308-1318.

CONTACT: *Suman Chakraborty; tel: +91-3222-282990; suman@mech.iitkgp.ernet.in