Centre for BioSystems Science and Engineering

THESIS COLLOQUIUM

at 11:00 AM on June 27, 2017
MRDG Seminar Hall

Investigations into the changes in biomechanics of liver cells upon Hepatitis C Virus infection.

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We have investigated, for the first time, the changes in biomechanics of cells upon virus infections using Hepatitis C Virus (HCV) infection in liver cells as a model system. The fact that HCV infection causes a few proteins to traverse the nuclear membrane into the cytoplasm prompted a study of the mechanics of nuclei.

In order to examine nuclear morphology, we extended a known 2D edge-detection algorithm to segment nuclear boundaries from not only 2D images but also confocal 3D images. It was observed that nuclei of liver cells harbouring HCV proteins have enhanced projected area and volume with reduced ellipticity. Using computational modelling, we show that the observed changes in projected area and volume of the nucleus could be due to reduced stiffness of the nuclei and enhanced tension in cortical actin. These predictions were validated by Atomic Force Microscopy (AFM) and protein quantification techniques in molecular biology.

Using AFM we show that the stiffness of liver cells harbouring HCV proteins is higher than control cells whereas their nuclei are significantly more flexible. Since the major component of cell stiffness is cortical actin, enhanced stiffness suggests higher tension in cortical actin. Furthermore, using western blot we show that liver cells harbouring HCV proteins express lower levels of Lamin A (protein that confers structural rigidity to the nucleus) and higher levels of actin in comparison to control cells. Over-expressing Lamin A in liver cells harbouring HCV proteins reduces the projected area of the nucleus but does not affect its ellipticity.

These alterations in nuclear mechanics might cause aberrant response of the nucleus to forces acting on liver cells. Since liver cells undergo copious amount of blood perfusion, we further investigated the consequences of shear stress due to flow on the nucleus. We subjected the cells to high shear stress (1 Pa) using micro-channels and found that the nuclei of cells harbouring HCV proteins become increasingly elliptical and align perpendicular to the direction of flow in comparison to control cells.

In order to ascertain whether the nucleus is susceptible to shear stress even at physiological levels (0.1 mPa), we built a perfusion culture system capable of growing cells under low shear stress for long durations. The system consists of miniature bioreactors, miniature peristaltic pumps and an integrated electrical control circuit. The bioreactors can incorporate micro-fabricated scaffolds and are compatible for high-magnification, live-cell imaging. By using miniature peristaltic pumps, we were able to place the entire system into the incubator for long-term culture. The system is scalable and in its present form can accommodate up to four independent, simultaneous, perfusion cultures. Using this system, we show that even under physiological shear stress, the nuclei of cells harbouring HCV proteins become increasingly elliptical over time (3 to 5 days) in comparison to control cells.

In summary, we have designed and fabricated devices, developed algorithms, used computational modelling, and performed experiments to discover a hitherto unknown deregulation of the nuclear mechanics of liver cells due to HCV infection. The nucleus becomes more flexible and susceptible to shear stress due to flow. We also show that these alterations in the mechanics of the nuclei are due to down-regulation of Lamin A. It is known that nuclear shape affects gene expression and disruption of the nuclear lamina can lead to gross changes in chromatin dynamics and regulation. Hence, our results suggest that some of the pathogenesis of HCV could be due to global changes in gene expression by deregulation of nuclear morphology and mechanics.

Poster presentation depicting short snippets from this work will start from 10:30 AM

For more information and artwork depicting the research (http://www.be.iisc.ernet.in/seminars.html)