学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（工学）　氏名　DO Tien Dung

学 位 論 文 題 名

Study of mechanical properties and cyclic stretching-induced remodeling of cellular primary cilia

(細胞一次繊毛の力学特性と繰り返し引張刺激に伴うリモデリングに関する研究)

The primary cilium is a solitary, immotile organelle that projects from the surface of almost every cells in the human body. It functions as a mechanosensor, which helps cells to sense surrounding mechanical signals such as fluid flow shear stress, and then adapt to the change of mechanical stimulation. Understanding the mechanical properties of primary cilia provide the better insight of how primary cilia response to applied mechanical signals. However, the mechanical properties of primary cilium is still not well-understood, especially the elastic and viscoelastic properties. Moreover, although the responses of primary cilia in response to fluid flow is well studied, the remodeling of primary cilia in response to indirect stimulation such as substrate stretching is a mystery. In this dissertation, the mechanical properties of primary cilia was elucidated, such as Young's modulus and viscoelastic properties, together with the remodelling of primary cilia in response cyclic substrate stretching.

Chapter 1 provides the fundamentals of cells, the primary cilium, its structures and the importance in cell life. The literature review of measurement of mechanical properties of cilia and remodelling function in response to mechanical forces were reviewed. The research purposes were also summarized, together with the relating technical issues.

Chapter 2 focuses on the measurement of mechanical properties of properties of primary cilia (Young's modulus, viscoelasticity) using micro-tensile test. Most of the prior studies investigated the flexural rigidity of primary cilia. As far as we know, this is the first study measuring directly the Young's modulus of isolated primary cilia. An in-house micro-tensile test exclusively fabricated in this study to measure mechanical properties of primary cilia, but it is able to modify for further investigation with the similar studied objective. The isolation technique, which peels off primary cilia from cells before testing, were also revealed. The measured global Young's modulus of primary cilia were found and compared to the local Young's modulus investigated by AFM to show confidently the measured values of both methods. Different strain rates were executed on isolated primary cilia, and the results showed the dependence of primary cilia Young's modulus at strain rates, which suggests their viscoelasticity. The viscoelastic properties of primary cilia was also elucidated by doing global fitting the mathematical model of primary cilia and the experimental results. The viscoelastic properties of primary cilia was modelled by standard linear solid model.

Chapter 3, for the first time, identifies the remodelling of primary cilia in response to mechanical signals of cyclic substrate stretching. Fluid flow shear stress exposed directly to primary cilia could be understandable to remodel primary cilia. However, whether and how the substrate stretching, a mechanical stimulus at the cell substrate, is capable of affecting primary cilia, sitting on the top of cells, is still mystery. Mechanically, primary cilia's base connects to the actin network, and the actin network also connects to the focal adhesions at the cell substrate, therefore, it is hypothesized that the actin network can be responsible for the cilia remodelling under cyclic substrate stretching. Experimentally, cells were seeded on the chamber and stretched at different strain levels and different amount of time. The results revealed that primary cilia may adapt and remodel their behaviours (length, incidence, orientation) in response to cyclic substrate stretching. By inhibiting the activity of actin network, it found that the actin cytoskeleton has huge impact on the cilia remodel. However, the underneath mechanism is still not clarified and need further investigations.

Chapter 4 shows the microstructure components of primary cilia using transmission electron microscopy (TEM), which provides a better understanding of responses of primary cilia in response to mechanical signals, and how these components contribute to cilia mechanics. The TEM images shows visually the connection of the base of primary cilia to the actin filaments supporting the hypothesis in Chapter 3 that actin network is an intermediate factor to transmit mechanical signal from the substrate to cilia. In addition, the numerical result in this chapter, for the first time, show a primary cilium model including completely the mechanical components of cilia: microtubules and cross-linking structures. Most of the previous studies assumed the primary cilium as a homogenous, elastic beam. This model may help further cilia simulation to obtain better understanding cilia mechanics.

Chapter 5 summarizes the works in the dissertation and offers the prospective studies that can be investigated in this research field.