Atomic force microscopy as a tool to study *Xenopus laevis* embryo

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Atomic force microscopy as a tool to study *Xenopus laevis* embryo

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Abstract. Atomic force microscopy (AFM) has become a powerful tool for imaging biological structures (from single molecules to living cells) and carrying out measurements of their mechanical properties. AFM provides three-dimensional high-resolution images of the studied biological objects in physiological environment. However there are only few AFM investigations of fresh tissue explants and virtually no such research on a whole organism, since most researchers work with cell cultures. In the current work AFM was used to observe the surface of living and fixed embryos and to measure mechanical properties of naive embryos and embryos with overexpression of guanine nucleotide-binding protein G-alpha-13.

1. Introduction

Cell- and tissue-scale mechanics integrates numerous processes within the multicellular organism from local cell movements to global tissue sculpting during morphogenesis. However, so far only few quantitative analyses of tissue and embryo biomechanics have been carried out [1, 2]. The challenge is to learn how gene products actually generate or modulate forces and provide cells and tissues with mechanical properties to transmit these forces in appropriate patterns. Recently many methods for measuring forces at molecular and cellular levels have been developed and atomic force microscopy-based indentation is one of them [3, 4, 5]. This method has a good resolution and requires rather simple sample preparation procedure.

The embryos of *Xenopus laevis*, a South African clawed toad, are frequently used in biological research because of the large embryo size and simplicity of manipulation [6]. We used AFM to study morphology of embryo surface and to carry out force measurements.

2. Materials and methods

In our previous work [7] we studied both fixed and living *Xenopus laevis* embryos with AFM, in order to visualize different stages of its development and to observe dynamic structure changes. We have developed a special sample preparation protocol for the observations. Embryos were placed and tightly

held in the holes made in agarose layer in such a way that the region of interest (e.g. animal or vegetal pole) was turned up. Such immobilization method did not affect the embryo viability, since the embryos could develop normally after prolonged (5–6 hours) experiments. Imaging was performed in contact mode in buffer solution or cultivation medium.

For the force measurements special cantilevers modified with 9 μm microspheres were used (figure 1). Such modification led to precise control of probe geometry and at the same time provided an estimate of the mechanical properties averaged over a relatively large contact area [5]. This setup allowed us to carry out measurements similar to those made with NanoNewton force measurement device described in [8].

The elastic (Young's) modulus of embryo was determined using Hertzian contact mechanics. For the naive embryos the measurements were carried out from blastula to neurula stages and for the embryos with overexpressed G-alpha-13 protein – only at gastrula stage.

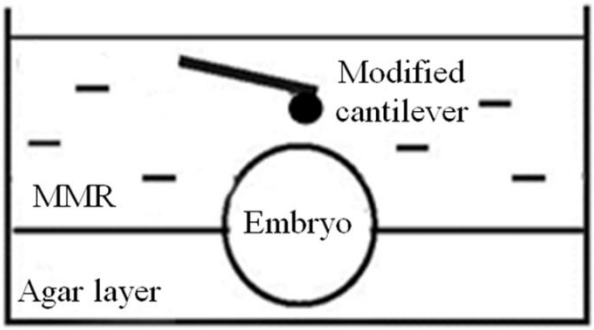

Figure 1. Scheme of experimental setup for force measurements of *Xenopus Laevis* embryos.

3. Results and discussions

3.1. Imaging the surface of *Xenopus Laevis* embryos

As described earlier [7], AFM can be used to image the embryos surface at various stages of development. AFM data were compared with the data from scanning electron microscopy (SEM). AFM investigation of fixed embryos allowed us to obtain data similar to SEM. We have also shown that AFM can be applied to study living embryos and visualize cell movements on the embryo surface *in vivo* in real time. The measured rate of elongation or shortening of the cell boundary fragments was ~0.5-1.5 μm/min.

The changes of microvilli quantity and distribution on the cell surface and the cytoskeleton remodelling were also observed (figure 2). In the area, indicated by box 1, cytoskeleton is clearly visible as network on the surface. The area, indicated by box 2, is rather smooth on the first image and some microvilli-like protrusions appear on the second image. Since AFM can be used to study living embryos, it can also be used to monitor the impact of bioactive compounds on embryogenesis.

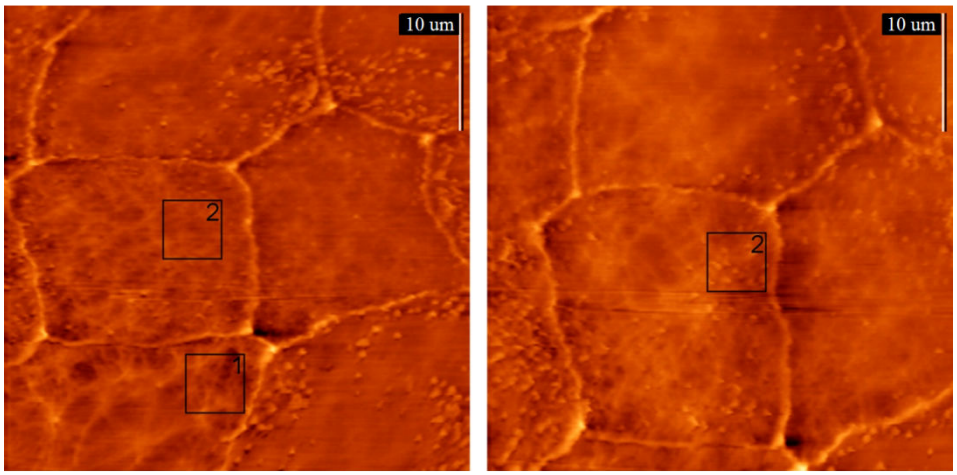

Figure 2. Two consecutive contact error images (~5 min on each scan) of living embryo surface (gastrula stage). The microvilli and cytoskeleton rearrangements are visible. In the area, indicated by box 1, cytoskeleton is clearly visible as network on the surface. In the area, indicated by box 2, some microvilli-like protrusions appeared on the second image.

3.2. Force measurements of *Xenopus Laevis* embryos.

Apart from imaging of the embryo surface AFM allowed us to measure the Young's modulus of the living embryo. The force measurements were carried out on living embryos during development from blastula to neurula stages. Our measurements of more than five embryos of each stage showed that there is a strong natural variability in stiffness among the embryos collected from the same or different females (1–200 kPa). Using our technique of immobilization of living embryos we managed to measure the stiffness properties in different embryo regions. On the gastrula-stage embryos we found that Young's modulus on the animal pole was more than two times higher than on the vegetal pole. Our data show the increasing of the embryos' elastic modulus (Young's modulus) on the animal pole during gastrulation. These phenomena can be explained by the fact of turgor blastocoel pressure growth, which stretches the blastocoel roof and may lead to rise of actin polymerization [9].

AFM is a convenient tool to study pathways of actin polymerization and regulation of cell stiffness. In our experimental model we measured the Young's modulus of G-alfa-13 overexpressing embryos under lysophosphatidic acid (LPA) treatment. LPA is a ligand of G-protein-coupled receptor [10]. For the downstream signal transduction LPA receptor uses heterotrimeric G proteins. LPA receptor activation leads to the structural change of the G protein and to the dissociation of its alpha subunit. It was showed that proteins of G-alpha-12/13 family can regulate actin cytoskeleton reorganization and cell motility. G-alpha-13 protein through RGS-RhoGEF complexes, such as LARG, p115Rho-GEF, PDZ-RhoGEF, leads to small GTFase RhoA activation which controls the actin polymerization. Thus, the signal cascade G13– RGS-RhoGEF– RhoA regulates actin cytoskeleton state [11, 12].

Numerous studies have proved the actin cytoskeleton to be the major contributor to the mechanical properties of single cells [13], however little is understood about the contribution of the actin cytoskeleton into mechanical properties of tissues. We found a significant growth of Young's modulus (about 100%) of the embryos with G-alpha-13 overexpression after addition of LPA into the cultivation medium (Figure 3). Apparently, LPA-activated signal cascade G13 – RGS-RhoGEF – RhoA induces the RhoA activation in the cell cortex of Xenopus embryos and increases its stiffness. Addition of LPA to ordinary embryos (without G13 overexpression) caused an increase of Young's modulus which did not exceed 15%.

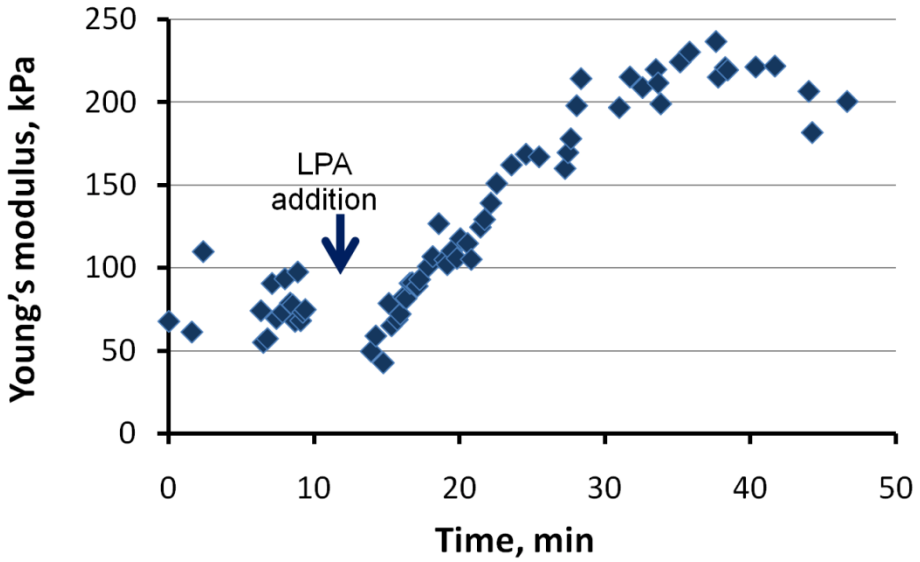

Figure 3. Growth of the Young's modulus of embryo with overexpression of G-alpha-13 protein after LPA addition.

4. Conclusions

We have shown that AFM is a powerful and convenient tool to study living embryos. We have detected an increase of Young's modulus upon impact of LPA on embryos with G-alpha-13 overexpression. Our findings allow us to recommend AFM as a method for studying morphology and stiffness properties of the different regions of the embryo during development.

Acknowledgments

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