

## 論文審査の要旨及び担当者

報告番号	(甲) 乙 第	号	氏 名	余 盈 君
<p>論文審査担当者 主 査 薬理学 安 井 正 人            医化学 末 松 誠 内科学 金 井 隆 典            血液浄化・透析センター 林 松 彦</p> <p>学力確認担当者： 審査委員長：末松 誠            試問日：平成25年12月 3日</p>				
<p>( 論 文 審 査 の 要 旨 )</p> <p>論文題名：Direct visualization and quantitative analysis of water diffusion in complex biological tissues using CARS microscopy            (CARS非線形光学顕微鏡を用いた水の直接観察に基づく生体組織における水拡散動態の定量的解析)</p> <p>Physiology of epithelial water transport is very important for understanding certain aspects of human disease. However, development of this area has been hampered by a lack of a direct measuring method of water movement. The present study showed that CARS microscopy combined with computer simulation succeeded to measure the diffusive water permeability (<math>P_d</math>) of individual luminal and basolateral water pathways in complex biological system such as 3D-epithelial cysts. This method is sensitive enough to measure <math>P_d</math> changes via hormonal regulation of aquaporin (AQP) expression in cysts.</p> <p>In the final thesis evaluation, several questions have been pointed out about this study:</p> <ol style="list-style-type: none"> <li>1) Why were, in 25% of H<sub>2</sub>O/D<sub>2</sub>O switching experiments, smaller values of <math>P_d</math> observed in case of D<sub>2</sub>O to H<sub>2</sub>O sequence, compared to H<sub>2</sub>O to D<sub>2</sub>O sequence? -- Whereas we could not identify the reason, previous studies suggested that D<sub>2</sub>O might have some effects on biological systems through unknown mechanisms. However 75% of experiments gave similar <math>P_d</math> values in both switching protocols, which implies that it should not cause a principal methodological problem.</li> <li>2) Which mechanisms underlie the baseline of diffusive water permeability of the membrane without AQP? -- It had been reported that artificial lipid bilayers have significant <math>P_d</math> of <math>2 \times 10^{-5} \sim 2 \times 10^{-3}</math> cm/s. Therefore, we suggested that the baseline of diffusive water permeability should be mainly mediated by the lipid bilayer component of the cell membrane.</li> <li>3) Have you characterized the heterogeneity of MDCK or M1 cells? -- We indeed found some heterogeneity, for example, in terms of AQP expression. To minimize the deviation originating from the cyst heterogeneity, we only used the cysts with an almost perfect spherical shape formed by monolayer of uniform thickness.</li> <li>4) What is the reason behind the twofold increase in basolateral <math>P_d</math> compared to luminal <math>P_d</math>? -- It should be due to the different membrane surface areas of basolateral and luminal sides; basolateral area is larger than luminal area.</li> </ol> <p>All the referees agreed that the current study of CARS microscopy combined with computer simulation made a significant improvement in measuring the diffusive water permeability in complex biological tissues, and that it is eligible as the PhD thesis, although a few more suggestions were made by the referees for further improvements of this study in future; siRNA/knockdown experiments for AQP, application to leaky type of cysts, and availability to high throughput drug screening.</p>				