

博士論文（要約）

上皮細胞の二方向観察可能な
多孔膜を有するガラスチップ

酒井 香苗

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本文

本論文は「上皮細胞の二方向観察可能な多孔膜を有するガラスチップ」と題し、5章から構成される。

上皮細胞層の構造異常とバリア機能の低下はガンや感染症といった種々の疾患原因となることが知られており、上皮細胞の形態観察とバリア機能の指標である透過性の評価は疾患のメカニズム解明の観点から研究が行われている。これまでの上皮細胞層の解析ツールは水平・垂直の二方向を直接形態観察することはできないということ、また透過性の評価には煩雑なサンプリングが必要といった欠点をもつ。本論文の目的は、上皮細胞層を垂直方向と水平方向の二方向から形態観察し、物質透過性評価をサンプリング無しに実施できるデバイスを実現することである。

第1章「序論」では、本研究の目的と意義、背景、従来研究について述べている。本論文の手法として、二つのコの字型ガラス流路（ガラスチャネル）で多孔膜を挟む構造を有するガラスチップを提案し、作製方針について述べている。

第2章「多孔膜を有するガラスチップの作製」では、ガラスチップの材料と作製手法の検討について述べている。セミオートマチックダイシングソーを用いてガラスキャピラリーを切断してガラスチャネルを作製し、 $0.4\text{ }\mu\text{m}$ の孔を有するPET膜（多孔膜）を上下両側から挟み込み、直径 0.5 mm のETFE絶縁電線をガイドワイヤーとして使用して、導入口に紫外線硬化光学素子用接着剤を用いてテフロンチューブを装着することでガラスチップを得る手法について詳細に記載している。

第3章「ガラスチップの評価」では、作製したガラスチップのPET膜がミスアライメントなく導入され、PET膜導入後も拡散機能を有するかどうかを確認している。さらにガラスチップ内部に導入された内液の蒸発や送液による漏れ出しについて評価を行っている。ガラスチャネルの接合部を紫外線硬化光学素子用接着剤でシーリングすることにより、ガラスチップの内液蒸発と送液による漏れ出しを低減可能であることを示している。

第4章「ガラスチップを用いた細胞観察・透過性評価とその応用」では、上皮細胞層の水平・垂直の二方向での形態観察と透過試験についての検討がなされている。ガラスチップ内でヒト胎盤バリアのモデルに使用される胎盤絨毛上皮細胞（BeWo細胞）を培養して細胞層を形成させ、水平方向と垂直方向の二方向観察が可能であることを示している。従来法で実施されている、細胞層の水平方向画像を多数枚取得して積層させて再構築した三次元画像

から取得した、細胞層の垂直方向の画像と、本チップを用いて一回のスキャンで獲得した細胞の垂直方向画像を比較し、解像度が従来法よりも向上していることを示し議論している。さらに、蛍光マーカー分子を用いた透過率測定を実施し、上皮細胞層の透過性評価への応用可能性を示している。

第5章「結論」では、本研究によって得られた結果に基づいて結論を述べるとともに、多孔膜を有するガラスチップについて今後の展望を述べている。

以上を要するに、本論文では、上皮細胞の二方向観察可能な多孔膜を有するガラスチップを作製する方法を確立し、作製したガラスチップについて上皮細胞層の形態観察と透過性評価試験への応用可能性を示した。

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