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Abstract of Thesis

Name （ZENG JINFENG）	
Title	Studies on Nanometer-sized Artificial Basement Membranes for Cell Compartmentalization in Three-dimensional Tissues (三次元組織内の細胞区画化のためのナノメートルサイズの人工基底膜に関する研究)

Abstract of Thesis

In this thesis, robust artificial basement membranes (A-BMs) based on nanometer-sized multilayered films were constructed by layer-by-layer (LbL) assembly technique, and their barrier ability to main cell compartmentalization in three-dimensional (3D) tissues was investigated.

Firstly, the fabrication of A-BMs was introduced based on type IV collagen and laminin multilayered nanofilms (Col-IV/LM) by LbL assembly approach, which are the main components of natural BMs, to imitate the structure and biofunctions of natural BMs *in vitro*. The simple operation and materials-versatility of the LbL assembly method enable the fabrication of nanometer-sized films with controllable components and thickness. The obtained multilayered Col-IV/LM nanofilms showed adjustable components, thickness, and porous networks, similar to natural BMs. Meanwhile, a wide range of surface roughness and Young's modulus of Col-IV/LM nanofilms were achieved by simply changing the concentrations of assembly solutions, allowing for their application as A-BMs in different tissues *in vitro*. Benefit by the specific interactions between Col-IV, LM and cell membrane, cell functions, including cell adhesion, proliferation, differentiation, were enhanced on A-BMs. Col-IV/LM nanofilms acting as a physical barrier permitted the compartmentalized coculture of fibroblasts and endothelial cells but allowed cell-cell crosstalk by molecular permeability through the porous networks. Furthermore, a robust A-BMs based on crosslinked Col-IV/LM nanofilms was developed by adding TGase on 3D fibroblast tissue. By *in-situ* cross-linking, the stability of A-BMs maintained between endothelial cells and fibroblast tissues was improved, contributing to the maintenance of cell compartmentalization for a longer time.

In order to further describe the possibility of Col-IV/LM nanofilms serving as A-BMs, the microstructure and biofunctions were compared with natural BMs.

Compared with natural BMs, the network density of Col-IV/LM Nanofilms was slightly lower, possessing a larger pore size and fiber diameter, which caused a higher molecular permeability (2.6 %) than the results *in vivo*. It is worth noting, however, that pores and fibers structure of nanofilms were observed at a dry state and that will vary with wetting conditions. Meanwhile, thanks to their versatility and simple operation, the thickness and Young's modulus of Col-IV/LM Nanofilms were controllable over a wide range to satisfy the requirements in different tissues. Although the obtained A-BMs could assist in preventing cell migration for up to 5 days which was much lower than the stability of natural BMs maintaining cell compartmentalization, the construction speed of Col-IV/LM nanofilms was much quicker than the self-development by cells/tissues. It has been reported that in the case of immortalized alveolar type-II epithelial cells cultured with Matrigel *in vitro*, the globular or fibrous deposits of laminin were fused into a mesh or a partly plugged mesh after 5 days of culture and a thin BM sheet formed after 10 days of culture. Therefore, A-BMs based on Col-IV/LM nanofilms will play an essential role in maintaining organized cell co-culture before the secretion of BMs components and assist the assembly and formation of regenerative BMs in 3D tissues.

Furthermore, shape-customized Col-IV/LM multilayered nanofilms were fabricated successfully in 3D tissues directly by the combination of the LbL assembly approach and inkjet printing. Patterned fibroblasts and endothelial cells co-culture were achieved by the assistance of shape-customized A-BMs, contributing to the construction of patterned 3D tissues *in vitro* and providing more reliable organized tissue models for evaluating drug efficacy, nanotoxicology, and implantation.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、三次元組織内の細胞区画化のためのナノメートルサイズの人工基底膜に関する研究論文である。

過去数十年にわたり，組織工学による 3 次元組織や臓器の構築が注目を集めてきた。しかし、3 次元的に細胞の位置を正確に制御できるようになったとしても、細胞培養期間中に細胞が移動してしまうため、組織化された細胞の位置は簡単に破壊されてしまう。複雑で整然とした組織を in vitro で再現することは、いまだに大きな課題である。パターン化された細胞の局在を制御することは、複雑な区画化された 3D 組織を構築する上で重要である。

人体では、基底膜（BM）が間葉系組織と内皮系組織（または上皮系組織）の区画化に大きな役割を果たし、複雑な器官構造を維持している。BM による細胞の区画化の技術は、組織化された 3 次元組織を作製するための強力な手法となる。本研究では、*in vitro* で区画化された 3 次元組織を構築するために、天然の BM の主成分である IV 型コラーゲン（Col-IV）とラミニン（LM）を用いて、交互積層法により人工基底膜（A-BM）を作製し、細胞の区画化技術の構築と課題、今後の展望についてまとめた。

以上のように、本論文は、人工基底膜の作製と三次元組織内での細胞区画化という新しい概念の創成に関する独創性と新規性に優れた研究内容である。よって本論文は博士論文として価値あるものと認める。