論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Construction of engineered extracellular matrix proteins for regulation of cellular functions
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第10814号, 授与年月日:2018年3月26日, 学位の種別:課程博士, 審査員:小畠 英理,粂 昭苑,白木 伸明,堤 浩,三重 正和
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第10814号, Conferred date:2018/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	Biological Information 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Engineering)
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

In the present, tissue engineering techniques have been developed and require effective materials. In order to form efficient biomaterials similar to natural counterparts, it is appealing to construct matrix mimicking a natural niche-like cellular microenvironment where cells interact with other cells, extracellular matrices (ECMs), and signaling molecules. Mimicking these interactions in desired biomaterials can facilitate the design regulation.

The aim of this thesis is to generate multi-functional ECMs providing proper environments for cell differentiation, proliferation, and functionalization. To achieve this purpose, two main experiments are designed; (i) Immobilization of growth factor to extracellular matrix (chapter 2) and (ii) Development of functional extracellular matrices by fusion of bioactive sequences (chapter 3 and 4).

The main strategy for designing ECM proteins relies on the combination of two units; a stable structural unit and an active functional unit. Elastin-like polypeptide $(APGVGV)_{12}$, (E), which can bind to hydrophobic surface is used as stable structural unit in all of experiments in this thesis. Then, various active functional units that regulate cellular functions are fused to structural units.

In chapter 2, growth factors are tethered to the ECM proteins for induction of cell proliferation even without addition of any soluble growth factors. Here, basic fibroblast growth factor (bFGF) tethered via electrostatic interaction to ECM protein are constructed. bFGF has a highly basic amino acid domain in its heparin binding region, so this property of bFGF is focused for tethering to the designed ECM (ERE-D20). For tethering bFGF, a polyaspartic domain (D20), 5 repeats of 4 aspartic acids and serine, is introduced to designed ECM, ERE, which consists of elastin-like polypeptide (E) and cell adhesive RGD sequence. Cell cultures on bFGF-tethered ERE-D20 are well attached and grown without addition of soluble bFGF. Addition of bFGF inhibitor prove that bFGF tethered to ERE-D20 induce cell proliferation. From these results, bFGF-tethered designed ECM via electrostatic interaction can be an alternative method to immobilize growth factor for construction of efficient multi-functional ECMs.

In chapter 3, N-cadherin which is well studied in neural cell-cell adhesion activity and has ability to induce neurite outgrowth, is focused for induction of neural differentiation from mouse induced pluripotent stem (iPS) cells. Here, N-cadherin binding peptide (SWELYYPLRANL; CBP) have been characterized for their capability to mimic N-cadherin in the neural differentiation. Due to this property, N-cadherin binding peptide (CBP)is fused to the structural unit (E) and called as E-CBP for construction of neural inductive designed ECM protein. The designed ECM protein, E-CBP, is shown to promote the neural differentiation of mouse iPS cells without exogenous neuro-inductive signals. Therefore, it is proved that CBPs have neural conversion activity similar to N-cadherin. It is suggested that CBP can function as an N-cadherin mimicking peptide for neuro-conversion.

Finally, the designed ECM proteins for astrocyte differentiation from mouse iPS cells are constructed. The designed ECM proteins (EI, EY, and EIEY) that contain structural unit (E) and active functional units, IKVAV (I) and YIGSR (Y), are constructed. Embryoid bodies (EBs) are transferred onto designed ECM coated plates and soluble ciliary neurotrophic factor (CNTF) and bFGF are added to culture medium. After 20 days culture, induction of astrocyte differentiation is evaluated. From these results, it is clarified that EI has the particular effects on astrocyte differentiation same as natural counterpart, Laminin, and the combination of EI and CNTF acts instructively for differentiation of mouse iPS cells to an astrocyte fate.

In conclusion, the engineered proteins constructed in this study for regulating cellular functions will be useful biomaterials for various applications such as regenerative medicine. In the future perspectives, more complex multi-functional ECMs can be developed for example, combining various synthetic CAMs sequence in material construction and also coupling of growth factors. These various combinations of CAMs sequence and growth factors within same ECM can be proposed the effective materials for tissue engineering.