学位論文の要約

題目　DNA based Photo-controllable Extracellular Matrix-like Scaffolds to Understand and Control Cell Behaviour

（DNA を用いた光制御細胞外マトリックス様足場による細胞行動の理解と制御）

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序論

The extracellular matrix (ECM), housing the cells in tissues and organs acts as a physical scaffolding for the cells as well as provides them with various biochemical and biomechanical cues for a number of cellular functions like morphogenesis, differentiation, migration etc. The ECM has a unique composition and topology and provides the cells with a dynamic, reversible and a synergistically regulated environment. The current existing techniques that mimic the extracellular matrix either provide static adhesions or non-reversible environment. Using the powerful molecular level toolkit of DNA nanotechnology and combining it with azobenzene photoswitches we created a non-invasively regulated reversible DNA based extracellular matrix scaffolds. These photocontrolled DNA based matrices have immense potential to help us understand the language of the extracellular matrix better and instruct cellular behaviour. We designed two different types of the extracellular matrix like scaffolds using DNA Nanotechnology – 1) DNA Polymer to reversibly control the ligand spacing (cell adhesion peptide) changes and 2) Three dimensional DNA nanotubes to reversibly control the matrix stiffness changes. Both these DNA based extracellular matrices are used as cell culture substrates to control cellular morphology, get insights about cell-matrix interactions, focal adhesion formation and understand the language of the extracellular matrix better.

1. Non-invasive Regulation of Cellular Morphology using a Photoswitchable Mechanical DNA Polymer

(光スイッチング可能なメカニカル DNA ポリマーを用いた非侵襲的な細胞形態の制御)

The extracellular matrix, residing the cells provides a dynamic and reversible environment. Spatiotemporal cues are essential when cells are undergoing morphogenesis, repair and differentiation. Recapitulation of such an intricate system with reversible presentation of nanoscale cues can help us better understand cellular processes and can allow precise manipulation of cell function *in vitro*. Herein, we formulated a photoswitchable DNA mechanical nanostructure containing azobenzene moieties and dynamically regulated the spatial distance between adhesion peptides using the photoswitchable DNA polymer with photoirradiation. We found that the DNA polymer reversibly forms two different structures, a relaxed linear and shrunken compact form, observed by AFM. Using mechanical properties of this DNA polymer, UV and visible light irradiation induced significant morphology change of cells between a round shape and spindle shape, thus provides a tool to decipher the language of the ECM better. Further, using HeLa and human mesenchymal stem cells, we found that the photoresponsive mechanical DNA polymer labelled with RGD peptide, which causes a displacement of the RGD ligands, in turn causes the change in the spreading of the cell, the organization of the F-actin filaments, and formation of focal adhesions. Our dynamic and photocontrolled reversible DNA polymer can be used as an efficient and effective tool for understanding many important phenomena such as cell morphogenesis, tissue repair, cancer metastasis, cell differentiation etc. The above-mentioned phenomenon are the basis of cell-cell and cell-substrate attachment. Such an extracellular matrix mimicking dynamic tool is very critical and beneficial in understanding and expanding our knowledge.

2. Three dimensional Photo-controlled DNA Nanotubes as Stiffness Tunable Matrices for Controlling Cellular Behaviour

(三次元光制御 DNA ナノチューブを用いた細胞挙動制御用マトリクス)

Cell behavior is determined by numerous cues of the extracellular environment like ligand spacing, nanotopography, matrix stiffness. Matrix stiffness changes occur in many biological processes like wound healing, tumorigenesis, and development. These spatio-temporal dynamic changes in the stiffness can cause significant changes in cell morphology, cell signaling, migration, cytoskeleton etc. In this work, we formulated photo-controlled stiffness tunable DNA nanotubes, which can undergo reversible changes in their conformation upon UV and VIS irradiation. When used as a substrate for cell culture, the photo-controlled DNA nanotubes can tune cell morphology of HeLa cells from a long spindle shaped morphology with numerous filopodia to a compact round morphology with less abundant filopodia like extrusions.

Such a photo-controlled nanosystem can give us deep insights into cell-matrix interactions in the native extracellular matrix caused by minute changes in the stiffness. This dynamic and photo-controlled reversible DNA nanotube like matrix can be useful for understanding the initial stages of the pathophysiology of many diseases and developmental processes extend our understanding of the ECM matrix. Such a dynamic stiffness tunable matrix is crucial for expanding our knowledge about various cell-matrix phenomenon.