including radical generator camphorquinone was prepared by electropinning, cross-linked in ethanol solution, and swollen in aqueous solution, which successfully produced swollen micro-fiber gel matrix. By regulating the photocuring conditions, elastic modulus of fiber gel sheet was well-tuned. Assessment of movement of 3T3 fibroblasts on the prepared gel sheets with different stiffness showed that cells extended well on the surface of the sheet and did not enter the inside of soft matrix (1.4 kPa), whereas cells got into the hard sheet (15.8 kPa) and finally reached to the bottom of the sheet 6 hours after seeding. This result suggested that 3-D cell mechanotaxis can be driven by matrix-stiffness dependent manner as well as the 2-D mechanotaxis. Mechanism of such 3-D mechanotaxis and possible manipulation of 3-D cell movement by the design of stiffness of gel sheet are discussed.

1PT232

Efficient manipulation of cell mechanotaxis: effect of the curvature of micro-elasticity boundary
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The directional cell movements induced by a mechanical gradient on a substrate surface, so-called mechanotaxis, are useful model phenomenon to investigate the mechanobiology of cell motility. So far, we have established the elasticity boundary conditions required to induce mechanotaxis of fibroblasts with certain reproducibility; sharp linear boundary with enough narrow width and enough high elasticity jump in the single-cell adhered interface on cell culture gels. However, the induction efficiency of mechanotaxis was significantly affected by the shape of moving cells, suggesting the importance of a shape interaction between cells and elasticity boundary. In this study, to assess the effect of the shape interactions on the induction efficiency of mechanotaxis, a curvature of the elasticity boundary was systematically designed with a custom-built mask-free photolithography using elasticity-tunable photocurable styrene derivative. Fixing the elastic gradient strength as 30~40 kPa/50 μm, the radius of curvature of boundary was designed as 50, 100, 250, 500, 750, and 1000 μm in both convex and concave shapes. Migration of 3T3 fibroblasts was observed on these boundaries and efficiency of induction of mechanotaxis was analyzed. As the result, highly efficient mechanotaxis was induced on the convex boundary with 100 μm in radius and on the concave boundary with 750 μm in radius. Interestingly, directional cell migration toward a softer region emerged on the concave boundary with 50 μm in radius for the first time.

1PT233

選択的フлуオライトイオンの変動を制御する人工組織への選択的注入
Selective Injection of Fluorescent Nanobeads into Caenorhabditis elegans

We present a novel selective injection of fluorescent nanobeads into Caenorhabditis elegans (C. elegans) based on bio-nanomanipulation technique for in-vivo single cell analysis. The nanomanipulation system was constructed under hybrid microscope. The hybrid microscope consists of the optical microscope (OM) and environmental-scanning electron microscope (E-SEM). It realizes detail analyses of biological specimens by fluorescent imaging by the OM, and nano-scale manipulation by the imaging of the E-SEM. Based on the bio-nano manipulation system, we propose a nano-injection method by the nanoprobe insertion for embeddedness of nanobeads. The nanoprobe was designed to have the four tips to fix single nanobead. It was fabricated by focused ion beam (FIB) process. The nano-scale probe size is considered to be important for minimal damage to the biological specimens. In this work, as a biological specimen, the C. elegans was used, because it is one of the important model organisms for various biological analysis. The nanoinjection technique was applied to embed a fluorescent nanobead into a specific cell inside C. elegans for in-vivo analysis. We inserted the nanoprobe into the body of C. elegans at a depth in approximately 6 μm. The size of fluorescent nanobeads was 500 nm in diameter. The proposed system is considered to be important for future nano-surgery system for life innovation using model organism.

1PT234

W/O ドロップレプリット内での酵素を用いたG テンパロ質共役受容体のリガンドアセイシステム

Yeast-based ligand assay system for detecting G protein-coupled receptor activation in water-in-oil droplets
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G protein-coupled receptors (GPCRs) are involved in many different cellular processes, and are targets of many therapeutic drugs. We developed a yeast-based ligand assay system for detecting GPCR activation in water-in-oil (W/O) droplets. Our system is based on linking between the genotype and the phenotype using W/O droplets. DNA library encoding peptides, in vitro transcription-translation and protein expression, and budding yeast cells expressing target GPCR are co-encapsulated into droplets using a microchannel. Yeast cells are genetically engineered to express GFP in response to ligand stimulation. The generated droplets were collected into a microcentrifuge tube and incubated to allow the gene translation and ligand stimulation. The droplets would be fluorescent when synthesized peptides stimulate GPCRs, enabling easy identification of ligand candidates. Using this system, we firstly demonstrated cell-free GFP synthesis in the presence of yeast cells in W/O droplets, indicating the presence of yeast cells did not inhibit protein expression. Next, we encapsulated yeast cells expressing Ste2p (an endogenous GPCR) and the gene of α-factor (ligand of Ste2p) in the droplets. After incubation, GFP-expressing cells were clearly observed in response to α-factor stimulation. The α-factor synthesized from the five-molecule DNA template in the droplets successfully induced GFP expression. Our system will provide an effective approach for the high-throughput screening of peptide ligands targeted at mammalian GPCRs by evolutionary molecular engineering.

1PT235

フローレ式栄養細胞バイオセンサーの開発とその脳内栄養測定への応用
Development of a flow-type lactate biosensor and its application to the measurement of lactate in the mouse brain
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Lactate biosensors which enable us to measure lactate concentration in blood and other body fluids are applicable to some medical fields such as sports medicine. We fabricated a lactate biosensor by a flow-type sensing system, in which the injected sample flows into an immobilized enzyme (lactate oxidase; LOD) column. The column was fabricated by packing an immobilized enzyme support into a 30 mm long column. The support was obtained by immobilizing LOD on silica particles (particle size: 30-60 mesh). The immobilization was performed by loading LOD onto the amidoalkyl-bonded particles with glutaraldehyde. The column was kept at a constant temperature (37°C). The generated H2O2 or consumed O2 in the column was detected by the H2O2 electrode or O2 electrode. The fabricated biosensor could measure lactate concentration up to 10 mM. By using it, we next measured lactate levels of the mouse brain. The samples were prepared by homogenizing the brain slices in AC5F. The results showed the differences of lactate levels among each part of the brain and among each age of the mouse.

1PT236

マイクロチャンネル内でのβ-グルコシダーゼの酵素活性のモニタリング
Monitoring the single-molecule enzymatic activity of β-glucosidase in a microchannel array chip
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β-glucosidase (BGL), which catalyzes the hydrolysis of cellulose to glucose, is one of the important enzymes in bioethanol production. We aim to obtain BGL mutants with improved hydrolytic activity by directed evolution. To screen many potential mutants easily and precisely, we are trying to develop the single-