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学位論文題目	Development of Biomimetic Matrices from Cultured Cells （培養細胞由来のマトリックス材料の作製）

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論　文　の　要　旨

Cells are surrounded by the extracellular matrix (ECM) which plays an important role in regulation of cell functions and tissue development. The main function of ECM on cells is to provide biochemical and biomechanical signals that regulate cell behaviors such as cell attachment, proliferation, migration, differentiation and apoptosis. ECM is a dynamic and complex environment characterized by biophysical, biomechanical and biochemical properties specific for each tissue. The composition of specific matrices not only varies with the type of tissue, but also alters according to the tissue's development stage and pathological state. Therefore, precisely controlled ECM model is necessary for regulation of cell functions and effective regeneration of functional tissues.

A detailed characterization of ECM constituents is now considered essential for understanding cell behaviors in the context of tissue and organ development. Therefore, precisely controllable ECM model is required to figure out the role of ECM. Generally, chemical coating of ECM, tissue decellularization and cell-derived ECM are used to mimic the native ECM. However, chemical coating method is hard to achieve the molecular complexity of matrices. Although some decellularization tissues are used in pre-clinical research due to the preserved tissue architecture, most of the decellularization tissues are xenogeneic matrices that may have potential risks of pathogen transmission and provocation of undesirable inflammatory and immunological reaction. The decellularization tissue matrices cannot mimic the development process of the tissues either. Similar to decellularization tissues and organs, ECMs derived from cultured cells represent a complex meshwork with a composition and organization of native ECM, which can mimic the development process of tissues. The properties of cell-derived ECMs can be controlled by manipulation of specific stimuli making them suitable for specific application. Cell-derived

ECM also can obtain a tunable architecture by deposition of ECM on template surface.

Stem cells are one of the most promising cell sources for tissue engineering and regeneration. When stem cells differentiate into somatic cells, they pass through stepwise stages of maturation. During the stepwise differentiation, the ECM produced by the cells dynamically alters to regulate the stem cell proliferation and differentiation. The properties of cell-derived ECM, especially biochemical composition, are highly dependent on the source cell population. Meanwhile, as disease states are often reflected by alteration of cell phenotypes, investigation of the properties of matrices derived from diseased cell populations should provide favorable models for better understanding the interaction between cells and ECM during disease progression.

In this study, biomimetic stepwise matrices were prepared by cell culture method. At first, a novel type of ECM stepwise and simultaneously mimicking osteogenesis and adipogenesis was developed to mimic the dynamic change of ECM during bone tissue development by culturing human bone marrow-derived mesenchymal stem cells (MSCs). The effect of ECM on MSCs differentiation was further investigated. Secondly, osteosarcoma microenvironment associated cells (MSCs, fibroblasts, osteoblasts and MG63 cells) derived matrices were prepared and used for investigation of their effects on MSCs and cancer cell (MG63) attachment and proliferation. Finally, three-dimensional (3D) ECM scaffolds mimicking chondrogenesis of MSCs were prepared and the role of “stepwise chondrogenesis-mimicking 3D scaffold” on regulation of chondrogenic differentiation of MSCs was investigated.

1. Development of stepwise and simultaneous osteogenesis-co-adipogenesis-mimicking matrices

In normal developmental stages, the ECM surrounding bone marrow-derived mesenchymal stem cells (MSCs) is remodeled according to the different stages of differentiation. Abnormal ECM dynamics are documented in clinical studies of many diseases. For example, the ECM in pathological cases such as osteoporosis has different characteristics compared with the normal tissues. The ECM in osteoporosis cases has a mixture composition derived from osteogenesis and adipogenesis of MSCs. So far, ECM during either osteogenesis or adipogenesis has been examined to understand their roles in pathological cases which disrupt the balance of osteogenesis and adipogenesis. Although decellularized osteoporotic bone tissues have been used, the stages of osteogenesis and adipogenesis are different from each patient. Decellularized osteoporotic bone tissues are not suitable for the investigation of ECM roles in the diseases caused by disruption of bone homeostasis. To solve this problem, ECM models which possess the composition of both osteogenic and adipogenic states and the stages of osteogenesis and adipogenesis are strongly desirable. In this part, a novel type of extracellular matrices that could mimic the dynamic variation of ECM was prepared from simultaneous osteogenesis and adipogenesis of human bone marrow-derived MSCs. Four types of osteogenesis-co-adipogenesis-mimicking matrices were prepared and used to investigate their effect on the osteogenic and adipogenic differentiation of MSCs. The simultaneous osteogenesis and adipogenesis of MSCs were induced by culturing MSCs in the mixture medium of osteogenic medium and adipogenic medium for different culture period. The four types of matrices are early osteogenesis and early

adipogenesis (OEAE) matrices, early osteogenesis and late adipogenesis (OEAL) matrices, late osteogenesis and early adipogenesis matrices (OLAE) matrices and late osteogenesis and late adipogenesis (OLAL) matrices. The matrices had different compositions. They supported adhesion and proliferation of MSCs and showed different effects on adipogenic and osteogenic differentiation of MSCs. OLAE and OLAL matrices promoted osteogenic differentiation but not adipogenic differentiation of MSCs. OEAE matrices promoted adipogenic differentiation but not osteogenic differentiation of MSCs. OEAL did not promote either osteogenic or adipogenic differentiation of MSCs. The stepwise osteogenesis-co-adipogenesis-mimicking matrices will provide a novel tool for investigation of the ECM effects on stem cell differentiation and some diseases.

2. Investigation of the effect of osteosarcoma involved cells derived ECM on MSCs and cancer cell behaviors.

Tumors are made of multiple cell types and components. Altered ECM properties have been associated with numerous pathological conditions including cancers. In all disease cases, changes to the ECM are not simply symptoms of disease but are contributes to the pathogenic process. In cancer, alteration in ECM composition and organization has been implicated in the progression of malignant tumors. Cancer progression is regulated by a complex interplay of genetic and epigenetic changes, evolving interactions between tumor cells and the surrounding ECM. In this part, osteosarcoma microenvironment associated cells such as MSCs, fibroblasts (FB), osteoblasts (OB) and MG63 cells were cultured for preparation of cell-derived ECM (MSC-ECM, FB-ECM, OB-ECM and MG63-ECM). These ECMs showed different components and different effects on MSCs and MG63 cells attachment, spreading and proliferation. MSC-ECM and FB-ECM highly enhanced the initial attachment and spreading of both MSCs and MG63 cells. OB-ECM slightly enhanced the initial attachment of MSCs and MG63 cells. On contrast, MG63-ECM obviously inhibited the initial attachment and spreading compared to TCPS. The matrices will be useful for investigation of the effect of ECM on cancer cells functions.

3. Development of stepwise chondrogenesis-mimicking 3D matrices scaffold

Besides osteogenesis and adipogenesis, MSCs also can differentiate into chondrocytes. In this part, 3D stepwise chondrogenesis-mimicking ECM scaffolds were prepared from mesenchymal stem cells (MSCs) by controlling the stages of chondrogenic differentiation. ECM scaffolds mimicking the early stage and late stage of chondrogenesis were obtained when MSCs cultured in the chondrogenic medium for 1 and 3 weeks, respectively. The ECM scaffolds had different compositions. Stem cell (SC)-ECM scaffold was rich in collagen I and biglycan, early stage chondrogenesis-mimicking (CE)-ECM scaffold was rich in collagen II, while the late stage chondrogenesis-mimicking (CL)-ECM scaffold was rich in collagen II and aggrecan. These three ECM scaffolds had different effects on chondrogenesis of MSCs. The CE-ECM scaffold facilitated chondrogenesis, however, the CL-ECM scaffold remarkably inhibited chondrogenesis of MSCs.

These ECM scaffolds not only provide new 3D ECM models to investigate the effects of ECM on MSCs functions, but also can be used as favorable ECM scaffolds for tissue engineering.

In summary, cell-derived matrices were demonstrated to be a good alternative to investigate the interaction between cells and ECM and the role of ECM on regulation of stem cells. “Stepwise development-mimicking matrices” provided well controlled ECM models for mimicking the differentiation process of stem cells. According to the different cell populations related to the diseases, diseased cells ECM could also be obtained. In addition, cell-derived matrices not only could be deposited on two-dimensional substrates, but also could be formed in 3D ECM scaffolds by using 3D scaffold templates. All the matrices and ECM scaffolds could mimic the dynamic ECM variation during osteogenesis and adipogenesis of MSCs. The advantage of cultured cell-derived ECMs is their best controllability due to the easy availability and controllable culture conditions. Specific ECMs could be developed for regulating cell functions in a desirable way. Therefore, the future of cultured cell-derived ECM in tissue regeneration is promising.

審　　査　　の　　要　　旨

〔批評〕

本論文では、細胞機能を制御することを目的とし、分化段階の異なる細胞、及び異なる種類の細胞から生体模倣型マトリックス材料を作製した。まず、ヒト骨髄由来の間葉系幹細胞（MSCs）を細胞培養皿上で培養し、骨芽細胞と脂肪細胞への同時分化誘導を行った。骨分化誘導培地と脂肪分化誘導培地との混合培地の組成および培養時間を最適化することにより、「骨分化初期・脂肪分化初期」、「骨分化初期・脂肪分化後期」、「骨分化後期・脂肪分化初期」と「骨分化後期・脂肪分化後期」となるように細胞の分化誘導を行った。培養後、脱細胞処理することにより、細胞成分を選択的に除去し、骨分化と脂肪分化の度合いを制御した4種類の細胞外マトリックス材料（以下、マトリックス材料と記す）を作製した。得られた各マトリックス材料は異なる組成を有し、MSCs の分化に対して異なる効果を示すことが分かった。すなわち、「骨分化後期・脂肪分化初期」マトリックス材料と「骨分化後期・脂肪分化後期」マトリックス材料はMSCsの骨分化を促進したが、「骨分化初期・脂肪分化初期」マトリックス材料は MSCs の脂肪分化を促進した。「骨分化初期・脂肪分化後期」マトリックス材料は骨分化、脂肪分化のいずれも促進しなかった。また、骨肉腫に関わる MSCs、線維芽細胞、骨芽細胞及びヒト骨芽細胞株のMG63細胞を利用してこれらの細胞に由来する細胞外マトリックス材料を作製し、細胞外マトリックス材料による MSCs とMG63細胞の機能への影響を明らかにした。MSCs 由来のマトリックス材料と線維芽細胞由来のマトリックス材料は、細胞の接着と伸展をよく促進した。骨芽細胞由来のマトリックス材料は細胞の接着をやや促進した。一方、MG63細胞由来のマトリックス材料は細胞の接着と伸展を阻害した。これらの細胞由来のマトリックス材料は異なる成分で構成され、MSCsとMG63細胞の機能への影響はそれぞれ異なることが分かった。さらに、MSCsを三次元培養し、軟骨分化を段階的に制御することにより、幹細胞マトリックス足場材料、軟骨分化初期マトリックス足場材料と軟骨分化後期足場材料を開発した。幹細胞マトリックス足場材料はタイプ I コラーゲンを、軟骨分化初期マトリックス足場材料はタイプ II コラーゲンを、軟骨分化後期足場材料はタイプ II コラーゲンとアグリカンをそれぞれ豊富に含有していた。軟骨分化初期マトリックス足場材料は幹細胞の軟骨分化を促進したが、軟骨分化後期足場材料は幹細胞の軟骨分化を阻害した。本研究で得られた生

体模倣型マトリックス材料は、幹細胞の機能制御及び再生医療の研究において重要な学術的知見を与え、新しい機能性生体材料の開発に資するものである。よって、本論文は博士（工学）の学位論文として十分な学術的な価値をもつものと認める。

〔最終試験結果〕

平成27年2月13日、数理物質科学研究科学位論文審査委員会において審査委員の全員出席のもと、著者に論文について説明を求め、関連事項につき質疑応答を行った。その結果、審査委員全員によって、合格と判定された。

〔結論〕

上記の論文審査ならびに最終試験の結果に基づき、著者は博士（工学）の学位を受けるに十分な資格を有するものと認める。