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氏　　名	Mohammad Mosharraf Hossain（モハマド　モシャラフ　ホサイン）
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学位授与の要件	学位規則第４条第１項該当 工学研究科精密科学・応用物理学専攻
学位論文名	Carbon nanotube for differentiation of mouse stem cells and a non-invasive characterization method for cardiomyocytes （マウス幹細胞のカーボンナノチューブによる分化誘導と分化心筋細胞の非侵襲画像解析）
論文審査委員	（主査） 教　授　民谷　栄一 （副査） 教　授　河田　　聡　　教　授　小林　慶裕 医学系研究科教授　澤　　芳樹 北陸先端科学技術大学院大学マテリアルサイエンス研究科教授　高木　昌宏

論文内容の要旨

Embryonic stem cell (ES) and induced pluripotent stem (iPS) cells are getting increasing research focus due to their potentials in regenerative medicine. In order to use these cells in regenerative therapies, we need to differentiate them into desired types of cells, for example, beating cardiac muscle cells, neuronal cells or pancreatic islet cells etc. The differentiation of ES and iPS cell is a complex process of epigenetic modifications - the accomplishment of which needs physical, chemical or biological clues or a combination thereof. The differentiation process starts with the organization of ES/iPS cells into three dimensional aggregates popularly known as embryoid

bodies (EB). The EB formation and the subsequent treatments during EB plating and growths dictate their differentiation into diverse cell lineages. Proper and non-invasive characterization of the differentiated cells is essential for their clinical and research applications. In this research we characterized mouse ES cell derived contractile cardiomyocytes by non-invasive intensity variation based video image analysis method. On the other hand, a gravity-based, simple microfluidic platform for hanging drop embryoid body formation was developed and used for the formation of EBs form mouse ES and iPS cells. In order to modulate cell differentiation, the interaction between mouse ES cells and carboxylate-functionalized multiwalled carbon nanotubes (f-MWCNTs) have been also been studied by using two independent approaches. In the first approach, we used direct interaction of f-MWCNTs with mouse ES cells during EB formation stage and we studied cytotoxicity, cell proliferation, cell organization and differentiation under such interactions. We observed strongly beating cardiomyocytes in f-MWCNT exposed cells with traces of glia-like neuronal differentiation from f-MWCNT exposed EBs. In the second approach, we created a mesoscopically modified composite cell culture substrate by using f-MWCNT with gelatin. We observed the behavior of mES cells on this novel and nanomaterials-containing substrate. The substrate was found biocompatible and it promoted concurrent differentiation of mES cells into electro active cardiac muscle cells and neuronal cells. Different kinds of beatings were observed in differentiated cardiomyocytes. These findings will open up new opportunities for the use of nanomaterials in controlling the ES and iPS cell fate, for the applications of microfluidic in developing integrated cell aggregation and differentiation platforms and for easy characterization of differentiated cells in a non-invasive manner for the subsequent utilization of differentiated cells in clinical and non-clinical applications.

論文審査の結果の要旨

This doctoral thesis includes the study of mouse stem cell differentiation using carbon nanotubes with a novel characterization based on cardiac beating video analysis. It also includes the study on the use of microfluidic device for mouse stem cell aggregation which is the starting phase for differentiation. The major conclusions of the work are

1. A novel approach for easy, quick and non-invasive characterization of differentiated cardiomyocytes from mouse stem cells has been developed. The approach was successfully applied to estimate beating frequency, beating strength, number of beating patches and maturity or progression of beating in differentiating cells. Moreover, the successful applicability of the approach in measuring the response of differentiate cardiomyocytes towards inotropic agents was demonstrated.

2. The applicability of the carboxylate functionalized multiwalled carbon nanotubes (f-MWCNTs) in the quicker aggregation of mouse pluripotent cells resulting in bigger and more spherical embryoid bodies (EBs) without bodies has been shown. There were no visible toxicity form f-MWCNTs on cells as investigated by several approaches. The subsequent differentiation from such embryoid bodies showed that the resulting beating cardiomyocytes were beating at low frequency but with higher strength which indicated positive role of f-MWCNTs in modulating the mouse stem cell differentiation.

3. Mesoscopically modified composite cell culture substrate was created using composite of f-MWCNT and gelatin and the behavior of mouse stem cells on this substrate was evaluated. The substrate was found biocompatible and it promoted concurrent differentiation of stem cells into electro active cardiac muscle cells and neuronal cells. Different kinds of beatings were observed in differentiated cardiomyocytes.

4. A gravity-based, simple microfluidic platform for hanging drop embryoid body formation was developed and used for the formation of EBs form mouse ES and iPS cells. The platform resulted in uniform sized embryoid bodies which upon plating showed successful differentiation into cardiomyogenic lineage.

The studies reported in the thesis will open up new opportunities for the use of nanomaterials in controlling the ES and iPS cell fate.

Therefore we have no hesitation in recommending that the doctoral degree in the area of Applied Physics in particular Nano-bio engineering from our University is awarded to Mohammad Mosharraf Hossain on the basis of this dissertation.