Cell-Derived Matrix as Biomimetic Substrate for Cardiomyoblast Differentiation

Muhammad Suhaeri[1,2], In Gul Kim[1], Seyoung Van[1,2], and Kwideok Park[1,2,*]

[1]Center for Biomaterials, Korea Institute of Science and Technology, Seoul, Korea
[2]Dept. Biomedical Engineering, University of Science and Technology, Daejon, Korea

Statement of Purpose: Cells are surrounded by extracellular matrix (ECM), a complex microenvironment of many different macromolecules that provide specific chemical and physical cues.[1] In natural tissues, the cell-derived matrix (CDM) consists of diverse ECMs and plays an important role in the regulation of cell function and in the guidance of new tissue regeneration.[2] Previous studies have confirmed the effect of CDM for multi-lineage differentiation of human mesencymal stem cell. In this study, fibroblast-derived ECM (FDM) was investigated for *in vitro* culture of cardiomyoblast (H9C2) and for induction of cardiomyoblast differentiation.

Material & Methods: NIH3T3 mouse fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) for 5-6 days *in vitro* and then decellularized ECM using detergent and enzymes. FDM was compared against conventional culture platform, such as tissue culture plate, gelatin and ECM component fibronectin (FN). H9C2 cardiomyoblasts were cultured in growth medium (DMEM/10% FBS) until 70% confluence (2-3 days). The cardiomyogenic differentiation of H9C2 was then induced by changing the serum concentration to 1% and daily supplementation of 50 nM retinoic acid (RA) for up to 7 days. Morphological analysis of H9C2 was assessed using F-actin (Life Technologies) immunostaining. Cell area and circularity were measured from immunofluorescent image of F-actin by using image J software (NIH). Cell proliferation was determined by CCK-8 assay. Focal adhesion of H9C2 was also examined using immunofluorescence of vinculin (Santa Cruz Biotechnology). In addition, cardiomyogenic differentiation of H9C2 was evaluated using cardiomyocyte markers expression, such as alpha-actinin (α-actinin, Abcam) and cardiac troponin-T (cTnT, Abcam). Differentiation efficiency was quantitatively determined using Image J.

Results: From morphological analysis, adherent H9C2 cells on FDM in growth medium (GM) show more circular morphology from day 1 to 5 but the morphology significantly changed into a flat and elongated shape upon the switch into differentiation medium (DM) at day 3 (Figure 1). In proliferation assay of H9C2, the result showed that cells proliferated well with time in GM but the numbers were rather constant in DM. When focal adhesion of H9C2 was assayed using immunofluorescence of vinculin, cells cultured on gelatin revealed a larger area of focal adhesion compared to FN or FDM in DM at early time point. Both FN and FDM gave similar focal adhesion area in DM from day 0 to 7. The differentiation of H9C2 was confirmed by the expression of positive cardiac markers (α-actinin and cTnT) (Figure 2A). Interestingly the cells on FDM exhibited much higher percentage of differentiation efficiency compared to that of gelatin and FN (Figure 2B). The difference was statistically significant.

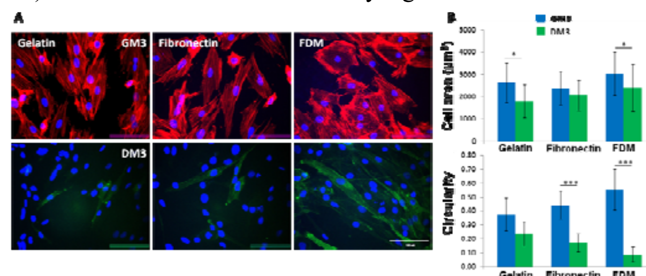

Figure 1. (A) Morphological observation of H9C2 at day 3 in different medium conditions. (B) Quantitative analysis of cell area and circularity (red, F-actin in GM; green, α-actinin in DM).

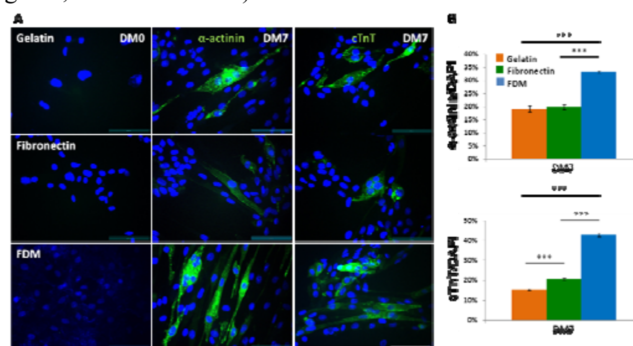

Figure 2. (A) Immunofluorescence of cardiogenic marker expression and (B) differentiation efficiency of H9C2 as determined by positive signals of cardiomyocyte markers (α-actinin and cTnT), both appeared in green.

Conclusions: Celluar behavior of cardiomyoblast, such as cell morphology, adhesion, proliferation, and differentiation is regulated by the context of substrate microenvironments. This preliminary study indicates that decellularized ECM can be a promising resource over gelatin or FN in the induction of cardiomyoblast differentiation. Further study includes ECM tuning to see the effect of matrix stiffness on cell differentiation.

References:

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