Efficient differentiation of human ES and iPS cells into cardiomyocytes on biomaterials under xeno-free

conditions

Tzu-Cheng Sung,^{†a} Cheng-Hui Liu,^{†b} Wei-Lun Huang,^b Yu-Chun Lee,^b S. Suresh Kumar,^c Yung Chang,^d Qing-Dong Ling,^{e,f} Shih-Tien Hsu^g and Akon Higuchi^{*a,b,d}

^aThe Eye Hospital of Wenzhou Medical University, No. 270, Xueyuan Road, Wenzhou, Zhejiang, 325027, China.

^bDepartment of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli,

Taoyuan, 32001 Taiwan

^cDepartment of Medical Microbiology and Parasitology, Universiti Putra Malaysia, 43400 Serdang, Slangor,

Malaysia

^dDepartment of Chemical Engineering, R&D Center for Membrane Technology, Chung Yuan Christian University, 200, Chung-Bei Rd., Chungli, Taoyuan, 320, Taiwan

^eGraduate Institute of Systems Biology and Bioinformatics, National Central University, No. 300, Jhongda RD.,

Jhongli, Taoyuan, 32001 Taiwan

^fCathay Medical Research Institute, Cathay General Hospital, No. 32, Ln 160, Jian-Cheng Road, Hsi-Chi City,

Taipei, 221, Taiwan

^gDepartment of Internal Medicine, Taiwan Landseed Hospital, 77, Kuangtai Road, Pingjen City, Taoyuan 32405, Taiwan

* Corresponding author. The Eye Hospital of Wenzhou Medical University, No. 270, Xueyuan Road, Wenzhou, Zhejiang, 325027, China, ROC.

Tel.: +86-0577-88017500; fax: +86-0577-88017508

E-mail address: higuchilb@yahoo.co.jp (A. Higuchi)

[†]These authors contributed equally to this work.

Supplementary Information



Supplementary Fig. 1 The sequential morphological changes during hESC (H9) differentiation on Matrigelcoated dishes towards the cardiac lineage. Morphologies of hESCs differentiated using Protocols 1, 2, 3, 4, and 5 on days 0, 2, 4, and 10 after induction of the cells into cardiomyocytes. Scale bar indicates 500 µm.



Supplementary Fig. 2 Characterization of hESC-derived cardiomyocytes. (a) Time dependent of survival ratio of the hESCs (H9) induced by differentiation into cardiomyocytes on Matrigel-coated dishes using Protocols 1, 2, 3, 4, and 5. (b) Time dependent beating colony numbers of the cells per well on 6 cm dishes, which were induced by differentiation into cardiomyocytes on Matrigel-coated dishes using Protocols 1, 2, 3, 4, and 5.



Supplementary Fig. 3 Immunostaining analysis of hESC-derived cardiomyocytes differentiated on several ECM-coated dishes using Protocol 2. Expression of cTnT on hESC-derived cardiomyocytes, which were differentiated on Matrigel-coated, Synthemax-coated, LN-511-coated, LN-521-coated, and rVN-coated dishes on day 14. Nuclei were stained with Hoechst 33342. Scale bar indicates 50 µm.



Supplementary Fig. 4 Characterization of hESC-derived cardiomyocytes differentiated on several ECMcoated dishes using Protocol 2. The α -actinin expression levels as analyzed by confocal laser microscopy on hESC-derived cardiomyocytes differentiated on the Matrigel-coated, Synthemax-coated, rVN-coated, LN-511coated and LN-521-coated dishes on day 14. Nuclei were stained with Hoechst 33342. Scale bar indicates 50 µm.



Supplementary Fig. 5 Immunostaining analysis of hESC-derived cardiomyocytes differentiated in several types of ECM-coated dishes using Protocol 4. Expression of α -actinin and MLV2c on hESC-derived cardiomyocytes analyzed by an immunostaining method in cells differentiated in Matrigel-coated, Synthemax-coated, rVN-coated, LN-511-coated, LN-521-coated, and FN-coated dishes on day 14. Nuclei were stained with Hoechst 33342. The scale bar indicates 100 µm.