TRPV-1 ACTIVATION THROUGH THERMAL AND AGONIST TREATMENT IN THE PROCESS OF SCALABLE CARDIAC DIFFERENTIATION AND TISSUES FABRICATION IS THE NOVEL STRATEGY TO ELIMINATE UNDIFFERENTIATED IPS CELLS IN THE BIOENGINEERED CARDIAC TISSUES

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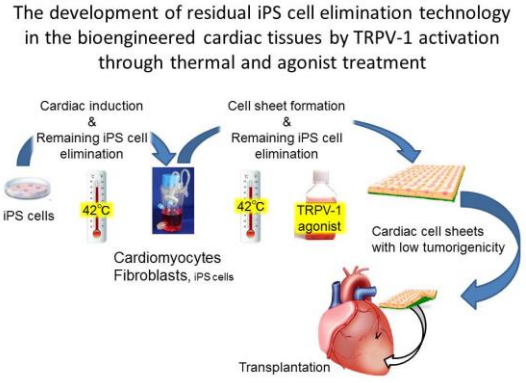
The replacement of injured heart tissues with the bioengineered cardiac tissues is expected as a possible therapeutic strategy for heart failure. Currently pluripotent stem cells are the most potent cell source for cardiac cells. However the risk of tumor formation due to the remaining undifferentiated stem cells in the bioengineered tissues remains resolved. We previously developed the scalable three-dimensional suspension bioreactor for human iPS cells and also the culture strategy for cardiac differentiation. Since some conditions including temperature, agitation rate, pH and dissolved oxygen concentration are continuously monitored and regulated in the bioreactor, it might be ideal to eliminate undifferentiated iPS cells in the process of cardiac differentiation with the optimization of these conditions. In the present study, we show that TRPV-1 activation through transient culture at 42 °C or with agonists is a simple and useful strategy to eliminate iPS cells from bioengineered cardiac cell sheet tissues. When feeder free human iPS cells were cultured at 42 °C, almost all cells disappeared by 48 hours through apoptosis. Furthermore when iPS cells were co-cultured with iPS cell-derived cardiac cells at 42 °C for 2 days, the number of Oct4 expressing iPS cells was significantly decreased. Conversely, in spite of cultivation at 42 °C, the number of iPS cell-derived cardiomyocytes and fibroblasts was maintained, and cardiac cell sheets were fabricated after reducing the temperature. TRPV-1 expression in iPS cells was upregulated at 42 °C, and the expression levels were significantly higher than that in cardiomyocytes, suggesting that iPS cells might be more sensitive to TRPV-1 activation than cardiomyocytes, which lead to eliminate iPS cells effectively without affecting cardiomyocyte viability. When cardiac cell sheets were cultured at 42 °C or with TRPV-1 agonist for 2 days, the expression of Lin28 and the number of Lin28 expressing cells were significantly decreased. Furthermore when 42 °C cultivation was applied to the later stage of cardiac differentiation in bioreactor, Lin28 expression was also significantly decreased. These findings suggest that the difference in tolerance to TRPV-1 activation between iPS cells and iPS cell-derived cardiac cells could be exploited to eliminate remaining iPS cells in bioengineered cell sheet tissues, which will further reduce the risk of tumour formation.



Reference
1) Sci Rep. 2016 Feb 18;6:21747
2) Tissue Eng. Part C 2015 Mar;21(3):330-8.
3) J Tissue Eng Regen Med. 2015 Jan 28. [Epub ahead of print]
4) Biochem Biophys Res Commun. 2012 Aug 24;425(2):321-7.