Fabrication of adipose tissue-derived cell sheets using charged thermoresponsive cell culture surfaces

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Statement of Purpose: For regenerative medicine using autologous cells, adipose tissue-derived cells (ADCs) are a promising supply source because adipose tissue can be harvested in large amounts with minimal invasion. ADCs are a cocktail containing various types of cells, including endothelial progenitor cells, pluripotent vascular progenitor cells (Lin K. Cytotherapy. 2008;10:417-426), and multipotent adipose-derived stem cells (ADSCs), which have the ability to undergo multilineage differentiation (Zuk PA. Tissue Eng. 2001;7:211-228). Therefore, ADCs have the potential to treat tissues because of their ability to differentiate and/or secrete cytokines (Schaffler A. Stem Cells. 2007;25:818-827). Typically, regenerative treatments using ADCs have been conducted via the injection of ADC suspension. However, the injection method has disadvantages, such as its inefficient implantation in the target tissue. In contrast to conventional injection methods, our laboratory developed an approach for efficiently transplanting cell sheets cultured on poly(*N*-isopropylacrylamide) (PIPAAm)-grafted cell culture surfaces (Kobayashi J. React Funct Polym. 2013;73:939-944). Cells cultured to confluence on hydrophobized PIPAAm-grafted surfaces at 37 °C are harvested as a contiguous cell sheet when the surfaces become hydrophilic as the temperature decreases to 20 °C. The extracellular matrix beneath the cell sheets easily attaches to tissue surfaces and maintains cell-cell junctions, leading to high transplantation efficacy. However, primary cells derived from tissues or organs, including ADCs, exhibit low adhesiveness to culture surfaces. Here, a new thermoresponsive cell culture surfaces is developed to enhance cell adhesion and ADC sheet formation. Introduction of charged moieties on the cell culture surfaces enhances the cell adhesion (Webb K. J Biomed Mater Res. 1998;41:422-430). However, the introduction of charged monomers into PIPAAm via copolymerization produces a large increase in the phase transition temperature of surface-grafted copolymer due to the increase in overall the hydrophilicity of random copolymer. To avoid the increase in the phase transition temperature of surface-grafted thermoresponsive polymers, thermoresponsive PIPAAm layers were separately grafted onto charged cell culture surfaces, which were utilized for enhancing ADC adhesion.

Methods: Positively and negatively charged thermoresponsive cell culture surfaces were prepared using commercially available BD PureCoat Amine and Carboxyl, respectively. These charged surfaces were modified with thermoresponsive polymeric layers by electron beam-induced grafting of *N*-isopropylacrylamide (IPAAm) solution in 2-propanol at a total concentration 55% (w/w). Primary mouse ADCs were obtained from the inguinal adipose tissues of wild-type mice as previously described (Ohashi K. Cell Med. 2012;3:113-119). ADCs were plated onto the thermoresponsive cell culture surfaces at 37 °C in a humidified atmosphere with 5% CO_2. Cell sheet detachment from the surfaces was performed by lowering temperature to 20 °C. Morphology and number of cells were monitored under a phase contact microscope at various time points.

Results: Relatively lager numbers of adhered ADCs after 24 h incubation were observed on both positively and negatively charged thermoresponsive surfaces than that on nonionic PIPAAm surface. More CD34-positive cells, a maker of adipose stromal cells and considered as pericyte and mesenchymal surface markers (Traktuev DO. Circ Res. 2008;102:77-85), adhered to both positively and negatively charged thermoresponsive surfaces than that on PIPAAm surface. After 12-day cultivation, the ADC sheet was harvested from negatively charged and nonionic thermoresponsive surface (Figure 1A and B), whereas the cultured ADC sheet was not able to detach itself from positively charged thermoresponsive surface due to strong adhesion onto the surface (Figure 1C.

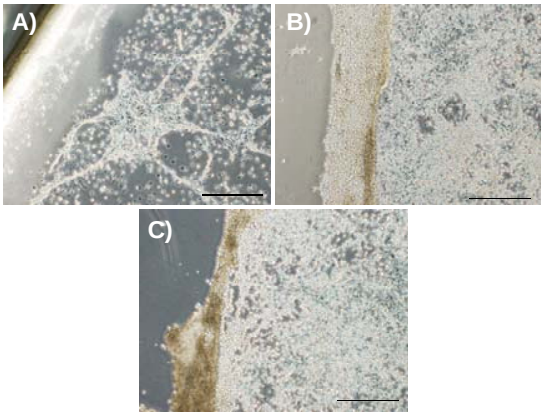

Figure 1. Detaching cultured ADC sheets from (A) positively charged, (B) negatively charged, and (C) nonionic thermoresponsive surfaces with lowering temperature to 20 °C. Scale bars: 500 μm

Conclusions: Charged thermoresponsive cell culture surfaces enhanced the adhesion of CD34-positive cells in ADCs. Fabrication of ADC sheets using charged thermoresponsive cell culture surfaces would be useful for efficient transplantation of adipose stromal cells onto the targets.

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