

Adipose-derived Stromal Cell Behaviors on Replicated Polymeric Lotus Leaf Structures

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Statement of Purpose: The cell-substrate interaction is influenced by surface properties including surface topography, chemistry, and chemical patterns. Therefore, surface characteristics of substrate play an important role in the cell behaviors. Recently, substrate topography representing the micro- and nano-sized structures has been reported to have significant effects on cell orientation, migration, morphology, proliferation and differentiation. Stem cells would be attractive to apply to substrate topography. Several studies began to report stem cell differentiation on patterned surfaces. However, substrate topography for stem cells expansion and differentiation has not been optimized. In addition, it is not clearly understood to elucidate mechanisms underlying between stem cell differentiation and surface property. In this study, we fabricated the replicated substrates with lotus leaf structures and investigated cell morphology and differentiation of adipose-derived stromal cells (ASCs) on them.

Methods: A nickel stamp with the lotus leaf structures was prepared by the nickel electroforming on the lotus leaf for the replication of substrates. The lotus leaf structures were molded on a polystyrene substrate via hot embossing processing. Before the hot embossing processing, the nickel stamp was treated with the trichlorosilane via vacuum evaporating for demolding between the nickel stamp and the embossed polystyrene substrate. The nickel stamp was heated up to 110°C. Then embossing was followed with a pressure of 3.2 MPa for 10 min. After cooling down the nickel stamp and the polystyrene substrate to 45°C, embossed substrate was demolded from the nickel stamp. Then the flat substrate and the lotus leaf structured substrate were treated with oxygen plasma (50 W, 1 mbar) for 10 s in order to increase the cell-substrate adhesion by increasing hydrophilicity of substrates. And these substrates were sterilized with ethylene oxide exposure. The ASCs were cultured on the flat substrates and the lotus leaf structured substrates, respectively. The cultured ASCs were stained with Calcein blue AM and observed morphology change under fluorescence microscope. In order to compare efficacy of ASCs differentiation on the substrates, the ASCs were cultured with adipogenic, chondrogenic and osteogenic induction media. Standard oil red O, Alcian blue and von Kossa stains were carried out to identify the differentiation of ASCs.

Results: Figure 1 shows scanning electron microscope (SEM) images of the lotus leaf structured substrate which is replicated with the nickel stamp via hot embossing. The replicated substrate is a multi scale structured surface which is the combination of micro pillars and sub-micro structures. The initial attachment rate of ASCs was slightly higher on the lotus substrate than on the flat substrate but proliferation was not significant (data not

shown). Figure 2 shows the cell morphology of ASCs on the flat substrate and the lotus leaf structured substrate after 3 days culture. The ASCs attached on the flat substrate widely spread in all directions, while they on the lotus leaf structured substrate showed relatively aggregative morphology resulting in smaller size of cells than on flat substrate. As ASCs differentiation in adipogenic media was observed, the lotus substrate induced higher adipogenic differentiation compared to flat substrate (Figure3).

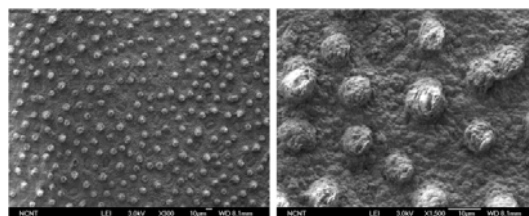


Figure 1. SEM images of the lotus leaf structured substrate.

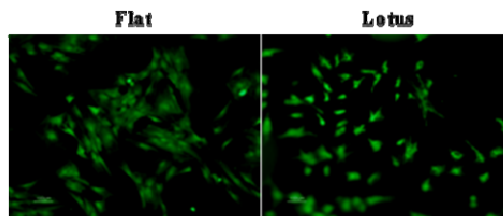


Figure 2. Fluorescence microscopic images of the ASCs morphology after 3 days culture.

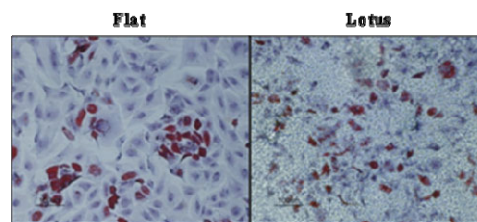


Figure 3. Oil red O staining of ASCs after 2 weeks culture.

Conclusions: The replicated substrates with lotus leaf structures were successfully fabricated by hot embossing processing. The ASCs tended to aggregate on the lotus leaf structures. Furthermore, the adipogenic differentiation of ASCs was significantly increased on the lotus leaf structures. These results reveal that the morphology and differentiation of the ASCs can be modulated by the lotus leaf structures. Further studies are required to clarify cell behaviors such as the focal adhesion, cell cycling and signaling.

References

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