發育再生研究快報

2011 Oct 14

Spheroid formation of mesenchymal stem cells on chitosan and chitosan-hyaluronan membrane

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Biomaterials 32(2011)6929-6945

ABSTRACT

Stem cells can lose their primitive properties during in vitro culture. The culture substrate may affect the behavior of stem cells as a result of cellesubstrate interaction. The maintenance of self-renewal for adult human mesenchymal stem cells (MSCs) by a biomaterial substrate, however, has not been reported in literature. In this study, MSCs isolated from human adipose (hADAS) and placenta (hPDMC) were cultured on chitosan membranes and those further modified by hyaluronan (chitosan-HA). It was observed that the MSCs of either origin formed three-dimensional spheroids that kept attached on the membranes. Spheroid formation was associated with the increased MMP-2 expression. Cells on chitosan- HA formed spheroids more quickly and the size of spheroids were larger than on chitosan alone. The expression of stemness marker genes (Oct4, Sox2, and Nanog) for MSCs on the materials was analyzed by the real-time RT-PCR. It was found that formation of spheroids on chitosan and chitosan-HA membranes helped to maintain the expression of stemness marker genes of MSCs compared to culturing cells on polystyrene dish. The maintenance of stemness marker gene expression was especially remarkable in hPDMC spheroids (vs. hADAS spheroids). Blocking CD44 by antibodies prevented the spheroid formation and decreased the stemness gene expression moderately; while treatment by Y-27632 compound inhibited the spheroid formation and significantly decreased the stemness gene expression. Upon chondrogenic induction, the MSC spheroids showed higher levels of Sox9, aggrecan, and collagen type II gene expression and were stained positive for glycosaminoglycan and collagen type II. hPDMC had better chondrogenic differentiation potential than hADAS upon induction. Our study suggested that the formation of

adhered spheroids on chitosan and chitosan-HA membranes may sustain the expression of stemness marker genes of MSCs and increase their chondrogenic differentiation capacity. The Rho/Rhoassociated kinase (ROCK) signaling pathway may be involved in spheroid formation.

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