Gonad tissue engineering by encapsulating differentiated germ cell-like cells derived from human endometrial stem cells (hEnSCs) in Fibrin Scaffold

Maryam Roya Ramzgouyan[1], Jafar Ai[1, 2]

1. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Brain and Spinal Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Recent studies in stem cells differentiation into germ cell-like cells has focused on infertility topics and reproductive problems. High potency of tumorogenicity and ethical issues of embryonic stem cells for clinical trials, have limited their applications. Instead, pluripotent cells isolated from adult tissues or organs could be a good alternative for gamete production. We identified the suitable conditions which promoted human endometrial stem cells (hEnSCs) differentiation into germ cell-like cells by regulating the concentration of retinoic acid (RA) in 2D medium. Afterwards, the differentiated germ cell-like cells were cultured in a 3D fibrin scaffold and their viability and properties were evaluated.

Materials and methods: Germ cell-like cells were derived from hEnSCs using an inductive medium containing RA in optimum concentration after 7 days. Then, characteristic cell markers such as Dazl, DDX4 and Dppa3 were determined by immunofluorescence and real time-PCR. Later, we encapsulate the hEnSCs-derived germ cell-like cells in fibrin gel and cell differentiation as well as viability were assessed by culturing for another 7 days. Structural and mechanical properties of the fibrin scaffold were examined with rheological analysis. Also viability of cells was analyzed using MTT assay.

Results: Immunohistochemistry and quantitative real-time PCR of germ cell-like cells differentiation markers showed that cell markers are expressed after 7 days culturing within fibrin matrix. Also, SEM analysis proved good cell integrity in the scaffold. MTT assay showed no toxicity effect of fibrin scaffold on the encapsulated cells.

Conclusion: It was found that hEnSCs were differentiated in retinoic acid containing medium and the germ cell markers were expressed properly in both 2D and 3D cultures. Also, fibrin gel could provide a suitable 3D scaffold for hEnSCs differentiated cells regarding tissue engineering of gonads.

.