9th Yazd International Congress and Student Award on Reproductive Medicine with 4th Congress of Reproductive Genetics

Award Winners

A-10
Mimicking the ovarian extracellular matrix, the role of natural polymrrs

Moshrefi M[1, 2], Jalali A[1], Ghasemi-Esmailabad S[2, 3], Samiei M[1, 4], Mollahoseini H[1, 5], Razban V[6, 7], Nikukar H[1, 8].

1. *Medical Nanotechnology and Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Marine Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.*
5. *Yazd Science and Technology Park, Medisa Polymer Ariya Company, Yazd, Iran.*
6. *Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.*
7. *Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
8. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: habibnik@ssu.ac.ir

Background: Female infertility treatment has been entered into a new world, constructed by Regenerative Medicine, which tries to get mature female gamete by help of the Tissue Engineering. So, ovarian follicles growth, ovarian in vitro activation, and ovarian follicle & tissue culture are the processes that can develop under the progress of tissue engineering. Development of ovarian follicles depends on endocrine and paracrine signals, the follicles micro-environment and 3-dimensional architecture. So, mimicking the ovarian extra cellular matrix by ovarian tissue engineering is a possible approach in fertility treatment for patients with premature ovarian failure and onco-fertility patients who cryopreserved the ovarian cortical tissue.
Objective: The current study aimed to assemble the electrospinning scaffolds by natural polymers, for comparison with collagen, as the natural ovarian tissue polymer.
Materials and Methods: After Ethical Committee permission, a full thickness section of human ovary from surface to hilum, was prepared. After chopping and enzymatic digestion by collagenase, the isolated cells were cultured. Besides, the electrospinning scaffolds were fabricated, using the natural polymers including agarose, human serum albumin, chitosan, collagen, silk fibroin, gelatin and a synthetic polymer (poly lactic acid (PLA)). Electrospun blended scaffolds of natural polymers with PLA in ratio of 30/70; 50/50 and 70/30 were prepared. Chemical properties of manufactured scaffolds were characterized by Fourier-Transform Infrared Spectroscopy analysis. Also, water contact angle was measured to quantify the surface wettability of the prepared scaffolds. Scanning electron microscope images analyses were performed before and after cell culture and the porosity and the average of fiber diameter distribution was calculated by ImageJ software. Cytotoxicity was evaluated by MTT assay after 14 days cell culture. Also, cell morphology and growth pattern was followed by hematoxylin and eosin staining.
Results: The blend of all natural polymers with PLA led to the fiber formation in electrospinning process, except for chitosan 70% + PLA 30%. Also, electrospinning for all the polymers separately led to fiber formation except chitosan and albumin. Therefore, 21 variables of electrospun scaffolds were assembled and Fourier-Transform Infrared Spectroscopy results confirmed the polymer accuracy. The results of fiber diameter diversity showed that the thickest fibers were related to the blended electrospun scaffold (50% agarose + 50% PLA) followed by (70% collagen + 30% PLA) ($\geq$ 200 nm). The other scaffolds fibers were below $\leq$ 150 nm. Compared to dish culture plate, MTT assay test after 4, 8 and 14 days cell culture on the scaffolds showed that the blends of (70% gelatin + 30% PLA) followed by (70% collagen + 30% PLA) led to the highest cell proliferation and the lowest cells toxicity, respectively.

Conclusion: Gelatin can be replaced by collagen, as the native extra cellular matrix of the ovary. Our results showed that gelatin, as an accessible natural polymer provided higher cell proliferation and lower fiber diameter than collagen. It is more accessible and cost effective with lower cell toxicity which makes it an optimized polymer for ovarian tissue engineering instead of collagen.

Key words: *Artificial ovary, Extracellular matrix, Natural polymers, Scaffolds, Poly lactic acid.*