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Poster Presentations

P-45

In vitro mouse spermatogenesis on artificial testis engineered by 3D printing of extracellular matrix

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Background: Male infertility accounts for about 50% of all infertility cases, and 25 % of infertile men are azoospermic. Due to the very small number of spermatogonia stem cells (SSCs) in testicular tissue biopsy specimens, SSCs culture for infertile patients can be important.

Objective: The proliferation of SSCs on printed scaffold derived from the extracellular matrix (ECM) of testicular tissues evaluated.

Materials and Methods: Ram testicular tissue was decellularized using hypertonic solution -Triton X-100

for 30 min. The extracted ECM (5% ratio) was used as a bio-ink for the fabrication of artificial testes along with alginate and gelatin. Testicular cells were then isolated from the testes of 3-7 days old neonate mice after enzymatic digestion. The nature of SSCs was confirmed by flow cytometry and RT-PCR for specific markers *Plzf*, *Id4*, *Gfra1*, and *Prml*. Finally, cell viability evaluated using MTT test and testicular cell proliferation process on printed alginate-gelatin scaffolds (group I) and ECM-alginate-gelatin scaffolds (group II) using immunocytochemistry, flow cytometry, and real-time PCR techniques was assessed.

Results: The MTT test indicated that the cell viability on the composite scaffold was significantly higher than the hybrid scaffolds and control group ($p > 0.05$). The results of 2 wk of proliferation on the printed system showed that the expression of *Plzf*, *Id4*, *Gfra1* gene using real-time PCR in group II was significantly higher than group I ($p > 0.05$). Flow cytometry analysis also showed that the number of *Plzf*-positive cells in group II was significantly higher than group I ($p > 0.05$). Immunocytochemistry results confirmed that *Plzf*, *Id4*, and *Gfra1* markers were expressed in both groups, but their expression in group II was significantly higher than group I ($p > 0.05$).

Conclusion: We concluded that the culture of testicular cells on scaffolds containing ECM increases the viability, colonization, and proliferation of SSCs and achieves a high number of cells for differentiation in vitro. Therefore, 3D printing using the ECM of the testis can be an ideal strategy for the regeneration of seminiferous tubules.

Key words: Spermatogonia stem cells, Extracellular matrix, 3D printing, Proliferation.