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Poster Presentations

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The effect of three-dimensional nanocomposite scaffolds on spermatogonial stem cells differentiation

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Background: Concern of azoospermia is common in male survivors of childhood cancer. Therefore, the culture of spermatogonial stem cells (SSC) for future treatment method is required, because these cells are important in spermatogenesis.

Objective: SSC isolation and differentiation are really important. 3D (Three-Dimensional) scaffolds play important role in cell culture, these scaffolds simulating a microenvironment similar to an extracellular matrix for differentiation of cells. The present study aimed to evaluate the efficiency of spermatogonial cells culture on a 3D microenvironment containing the Chitosan-Alginate (CA) scaffolds that contain graphene oxide (GO) nanocomposite for investigated the differentiation improving.

Materials and Methods: We isolated spermatogonial cells from neonatal 3- to 6-day-old NMRI mice. Then we prepared the scaffolds (Based on our last studies, which we added GO concentrations of 5, 15, 30, 45, 75 µg/ml to the CA, the seeded cells have shown strong attachment on CA/GO 30 µg and had the best biocompatibility compared with other concentrations. So, CA/GO 30 µg become our selected scaffold for this study). The scaffolds were analyzed using FTIR, XRD, and microCT to observe surface topography and morphology. SSC were cultured and divided in to 2 culture groups: (SSC + basic medium), and (SSC + CA/GO 30 µg scaffold). Basic medium was DMEM-F12 with KSR 10%, consisting of Bmp4 40 ng/ml and Retinoic acid 10-6 M. The stem cells related markers for differentiation of SSCs (*SYCP3* and *TEKT1*) were detected on all experimental groups by RT-qPCR and ICC.

Results: Incorporation of GO into CA matrix increased both crosslinking density as indicated by the reduction of crystalline peaks in the XRD patterns and polyelectrolyte ion complex as confirmed by the FTIR. MicroCT analyses indicate that the scaffold had a highly porous and interconnected pore structure with porosity of 81.56 %. The RT-qPCR results showed that the expression of *SYCP3* and *TEKT1* genes were higher after 14 days in the SSC + CA/GO group compared to control ($p < 0.05$). ICC assays results showed that the mean expression of *SYCP3* for SSCs cultured on the CA/GO 30 µg scaffold after 14 days was 49.37 ± 6.20 while Its mean expression for SSCs cultured on the basic medium after 14 days was 35.97 ± 4.70. The mean expression of *TEKT1* for SSCs cultured on the CA/GO 30 µg scaffold after 14 days was 73.12 ± 3.94 however this marker's mean expression for SSCs cultured on the basic medium after 14 days was 53.46 ± 3.09. So, the expression of *SYCP3* and *TEKT1* for (SSC + CA/GO 30µg scaffold) had significant increase than the other scaffold group ($p < 0.05$).

Conclusion: The most expression of differentiation markers was in CA/GO 30 µg group. This scaffold has biocompatibility and degradable properties and graphene oxide helps to strengthen the scaffold and thus improves cell culture. This scaffold will provide a more improved structural environment for increased differentiation of SSCs.

Key words: *Spermatogonial stem cells, Chitosan-Alginate Scaffold, Graphene oxide nanocomposite, Differentiation.*