**The effect of alginate-chitosan scaffold containing graphene-oxide nanocomposite on neonatal spermatogonia stem cell differentiation**

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**Abstract**

Boys who survived childhood cancer are at risk of azoospermia, a condition characterized by the absence of sperm in the semen. To address this issue, the isolation and purification of spermatogonial stem cells (SSCs) are crucial. Creating scaffolds that mimic the natural environment for these cells is essential for promoting their differentiation. A study aimed to assess the effectiveness of nanocomposite scaffolds made of alginate, chitosan, and graphene oxide (GO) in aiding SSCs differentiation. The scaffolds were tested for cytotoxicity using an MTT assay, and the sample containing 30 µg/mL of GO (ALGCS/GO30) showed the most promising results. Flow cytometry confirmed the identity of the cells using C-Kit and GFRα1 markers. Various analyses, including FTIR, XRD, and SEM, were conducted to evaluate the scaffolds' properties. The differentiation of SSCs was assessed using qRT-PCR. The results demonstrated that the ALGCS/GO30 nanocomposite scaffold was biocompatible, supported cell attachment, and promoted SSC differentiation. The scaffold showed a significant increase in differentiation markers compared to the control group. Immunocytochemistry confirmed higher levels of Sycp3 and Tekt1 protein expression in the nanocomposite scaffolds compared to those without GO. In conclusion, the biocompatible ALGCS/GO30 scaffold shows promise for promoting SSC differentiation in in vitro applications.

Keywords: spermatogonial stem cells; nanocomposite scaffolds; graphene oxide; alginate; chitosan