

**Conclusion:** Overall, the utilization of CRISPR/Cas9 in glioblastoma cancer stem cell research offers valuable insights into the molecular mechanisms underlying cancer stem cell maintenance and has exposed new avenues for targeted therapies. As this field continues to evolve, the integration of CRISPR/Cas9-based strategies with other emerging technologies will further enrich our comprehension of glioblastoma cancer stem cells and lead to more effective treatment strategies.

**Keywords:** Cancer Stem Cell, CRISPR/Cas9, Gene Editing, Glioblastoma, Targeted Therapies

**Ps-36: Effects of Conditioned Medium of Human Adipose-Derived Mesenchymal Stem Cells on Apoptosis of Endometriosis Cells**

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**Objective:** Although the pathogenic mechanisms related with endometriosis are not clearly understood, apoptosis seems to play a critical role. This research is to detect if Conditioned Medium (CM) obtained from human adipose tissue-derived mesenchymal stem (AD-MSCs) can promote apoptosis in Menstrual blood-derived stem cells (MenSCs) in women with endometriosis.

**Materials and Methods:** About 2 mL of menstrual blood collected from infertile women. MenSCs were cultured up to passage 3 and cell surface markers were analyzed by flow cytometry. To study the effect of CM on apoptosis of endometriosis cells, Annexin V assay followed by flow cytometry analysis was used. To further analyze the effect of CM on apoptosis and survival of endometriosis cells, mRNA expression of *BAX*, *Bcl-2* and *Survivin* was studied using real-time polymerase chain reaction (PCR).

**Results:** Apoptosis analysis by flow cytometry showed that late apoptosis and early apoptosis significantly increased in comparison with control group. The real-time PCR findings demonstrated that mRNA expression of *BAX* significantly increased as compared with untreated. In this study no significant differences were detected in mRNA expression of *Bcl-2* and *Survivin* between the groups.

**Conclusion:** The research demonstrate that CM could enhance apoptosis in endometriosis. Furthermore, the findings indicate that MenSCs may have a pivotal role in the pathogenesis of endometriosis and further validate the theory of endometriosis formation through retrograde menstrual blood flow.

**Keywords:** Apoptosis, Conditioned Medium, Endometriosis, Mesenchymal Stem Cells (MSCs), Menstrual Blood

**Ps-37: The Effect of Conditioned Medium Obtained from Human Adipose-Derived Mesenchymal Stem Cells on The Restoration of Spermatogenic Genes in A Rat Model of Azoospermia Induced by Busulfan**

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**Objective:** For men with non-obstructive azoospermia (NOA), assisted reproductive techniques (ART) are often the only viable option to have a biological child. This study aims to investigate

whether Conditioned Medium (CM) from Human adipose tissue-derived mesenchymal stem cells (AD-MSCs) can help to restore the expression of spermatogenic genes in a busulfan-induced azoospermic rat model.

**Materials and Methods:** To investigate the impact of CM intratestis injection on spermatogenesis recovery in a rat model of NOA, AD-MSCs were isolated and cultured. The CM was collected on the 3<sup>rd</sup> passage. The rats (30 male Wistar rats, aged 8-12 weeks) were divided into 4 groups: control group (no treatment), NOA group (busulfan-induced rats), azoospermia sham group (busulfan + Phosphate-buffered saline), and experimental group (busulfan + ADSC-CM). The NOA model was induced by intraperitoneal busulfan injections with a 21-day interval. After 35 days, the test group received ADSC-CM. Gene expression was analyzed using real-time polymerase chain reaction (PCR). **Results:** The analysis indicated a significant increase ( $P \leq 0.05$ ) in the levels of *DAZL*, *Miw* and *VASA* expression within the ADSC-CM group as compared to the control group, whereas a significant decrease was observed in the NOA and Sham groups. **Conclusion:** This study suggests that the use of CM obtained from AD-MSCs could potentially assist in the recovery of spermatogenesis in busulfan-induced infertile rats.

**Keywords:** Adipose Mesenchymal Stem Cells, Conditioned Medium, Non-Obstructive Azoospermia

**Ps-38: Human Three-Dimensional Acellular Amniotic Membrane Containing Adipose Tissue-Derived Mesenchymal Stem Cells; How Deep Is The Penetration of Allogenic Stem Cells into The Scaffold?**

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**Objective:** Simulation of *in vivo* conditions for mesenchymal stem cells in a laboratory environment and placing them on a biological ECM can not only improve the structural characteristics of stem cells but also significantly increase their secretory potential and function.

**Materials and Methods:** In this study, mesenchymal stem cells were extracted from adipose tissue, cultured until passage 3, and confirmed by flow cytometry and differentiation potential. In the next step, the amniotic membrane separated from placenta was decellularized using mechanical and chemical methods. According to the convergent scattering pattern, activated allogeneic mesenchymal stem cells ( $7 \times 10^5$  cells/each scaffold) were propagated for  $16 \pm 3$  hours at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  and 90% humidity. The structural, mechanical, biological, and physicochemical health of 3D scaffold was confirmed using SEM, MTT, FTIR and acridine orange staining methods. Safety and efficiency of 3D scaffolds were investigated in the healing process of diabetic wounds in C57 mice.

**Results:** We designed a three-dimensional biological scaffold composed of decellularized amniotic membrane with a diameter of 20-25  $\mu\text{m}$ , which contains active allogeneic mesenchymal stem cells derived from adipose tissue with a diameter of 1.8-2.8  $\mu\text{m}$ . Allogeneic mesenchymal stem cells not only settled and proliferated on the surface layer of acellular amniotic membrane but also permeated into deeper layers and formed a multilayered