

## #3345

## EFFECTS OF CONDITIONED MEDIUMS COLLECTED FROM 2D OR 3D CULTURED MSCS ON KIDNEY FUNCTIONS OF RATS WITH AUTOIMMUNE DIABETES INDUCED WITH STREPTOZOCIN

Serbay Ozkan<sup>1,2</sup>, Basak Isildar<sup>2,3</sup>, Hakan Sahin<sup>2</sup>, Halil İbrahim Saygi<sup>2,4</sup>, Dildar Konukoglu<sup>5</sup> and Meral Koyuturk<sup>2</sup>

<sup>1</sup>Izmir Katip Celebi University, Faculty of Medicine, Histology and Embryology, Izmir, Turkey, <sup>2</sup>Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Histology and Embryology, Istanbul, Turkey, <sup>3</sup>Balikesir University, Faculty of Medicine, Histology and Embryology, Balikesir, Turkey, <sup>4</sup>Istanbul Medeniyet University, Histology and Embryology, Istanbul, Turkey and <sup>5</sup>Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Biochemistry, Fatih, Turkey

**Background and Aims:** The main mechanism of therapeutic action of mesenchymal stem cells (MSCs) could be resulted from their secretome content rather than their direct differentiation into damaged cells [1, 2]. Preconditioning of MSCs with different strategies like culturing in 3 dimensional (3D) culture scaffolds could improve their therapeutic potential [1]. Based on our previous study [1] in which the concentrations of total protein and specifically VEGF and IL-4 in conditioned mediums (CMs) collected from MSCs cultured in 3D culture conditions were increased compared to one of 2D, the aim of this study is comparative analysis of effects of CMs obtained from MSCs incubated under 2D and 3D culture conditions on kidney functions of rats with autoimmune type I diabetes (aT1D) induced with streptozocin (STZ). **Method:** Isolation and characterization of the umbilical cord MSCs and the following analysis of concentrations of total protein and paracrine factors in the 2D-CMs and 3D-CMs were performed as it was mentioned [1]. Sprague-Dawley rats were treated with 20 mg/kg STZ for 5 consecutive days to induce T1D and 12 doses of CMs were intraperitoneally introduced for 4 weeks in

accordance with the timeline defined in Figure 1. Therapeutic effects of CMs were comparatively investigated by biochemical, physical, histopathological and immunohistochemical analysis.

**Results:** At the end of the 2<sup>nd</sup> week, urinary albumin/creatinine (u-Alb/Cr) ratio and creatinine removal rate (Crr) of diabetes were significantly aggravated compared to control group ( $p = 0,0001$  and  $p = 0,13$  respectively). At the end of the 7<sup>th</sup> week, u-Alb/Cr ratios of both treatment groups were significantly improved compared to one of diabetes group ( $p = 0,029$  vs  $p = 0,002$ ). There was no statistical difference among all groups for Crr values. In the post treatment period u-Alb/Cr ratio was exacerbated ( $p = 0,014$ ) while Crr value was not changed compared to pre-treatment period ( $p = 0,713$ ). There was no statistical change between the pre- and post-treatment values of u-Alb/Cr ratio and Crr for both treatment groups. Renal mass index of control group was lower than the ones of experimental groups, while weight percent change from pre- to post-treatment period of control group was statistically higher than the ones of experimental groups ( $p = 0,0001$ ). However, there was no statistical difference for both parameters among the experimental groups (Figure 2). Histopathological evaluations showed that cortical tubular damage (CTD) was significantly ameliorated in only D+3D-CM group compared to diabetes group ( $p = 0,012$ ), while there was no statistical change in glomerular fibrosis in both treatment groups (Figure 3.1 and 3.2 respectively). Immunohistochemical analysis indicated that nephrin expression was insignificantly increased in both treatment groups compared to diabetes group (Figure 3.3). Preliminary evaluation of thicknesses of glomerular basal membrane and pedicel with transmission electron microscopy (TEM) showed no clear difference among all the groups (Figure 4).

**Conclusion:** CM obtained from MSCs in 3D culture conditions could partially ameliorate kidney functions of aT1D by improving CTD and expression of nephrin which is crucial for the integrity of glomerular filtration barrier.

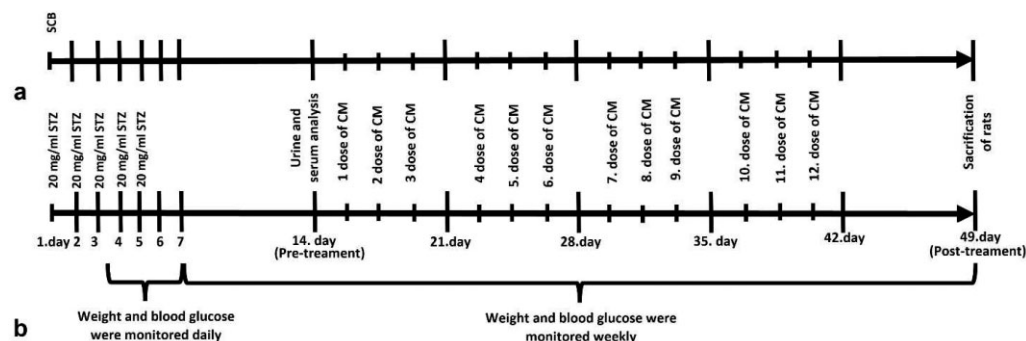
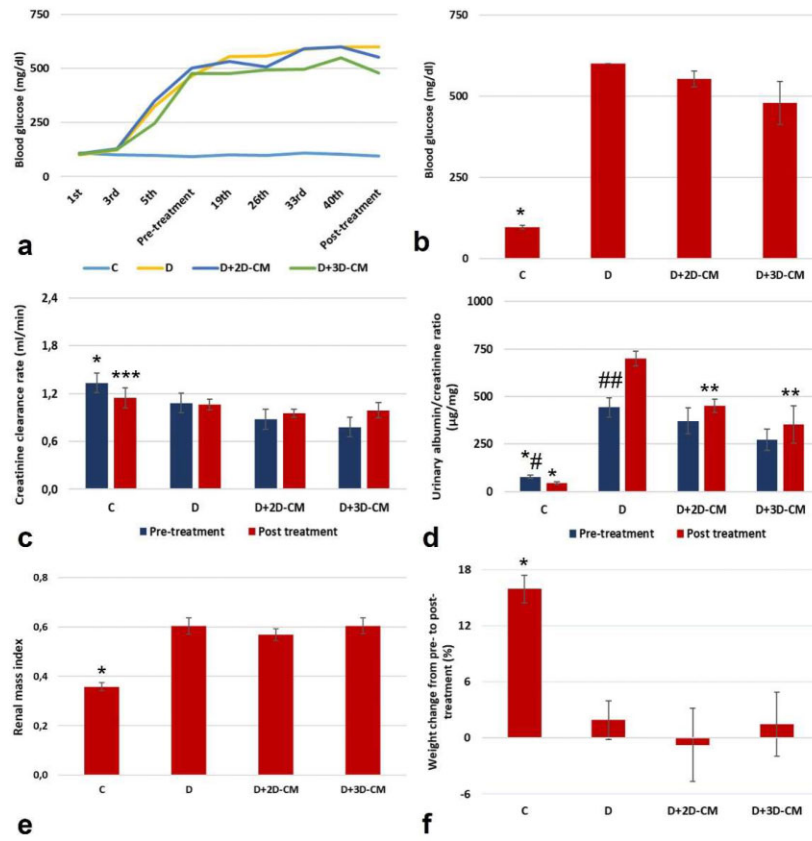
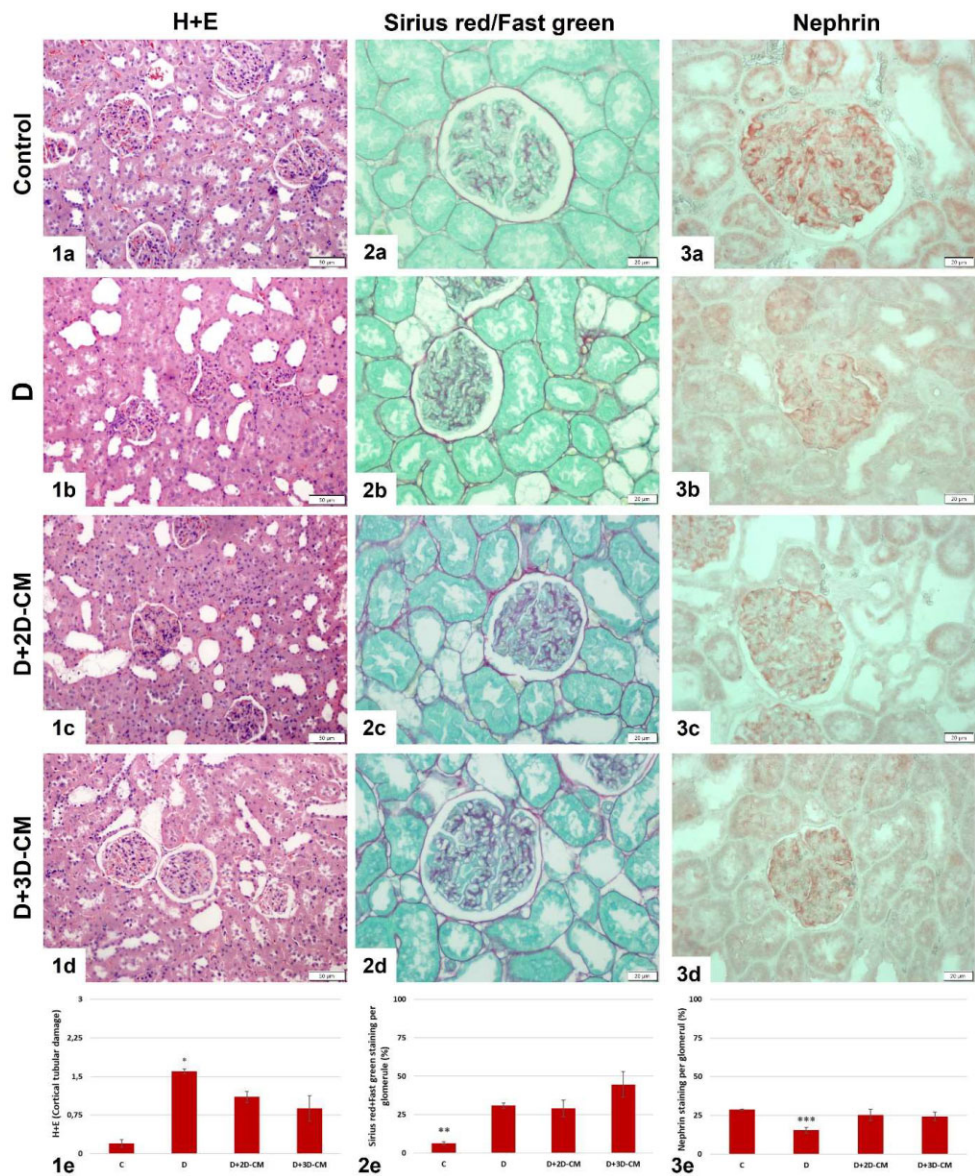


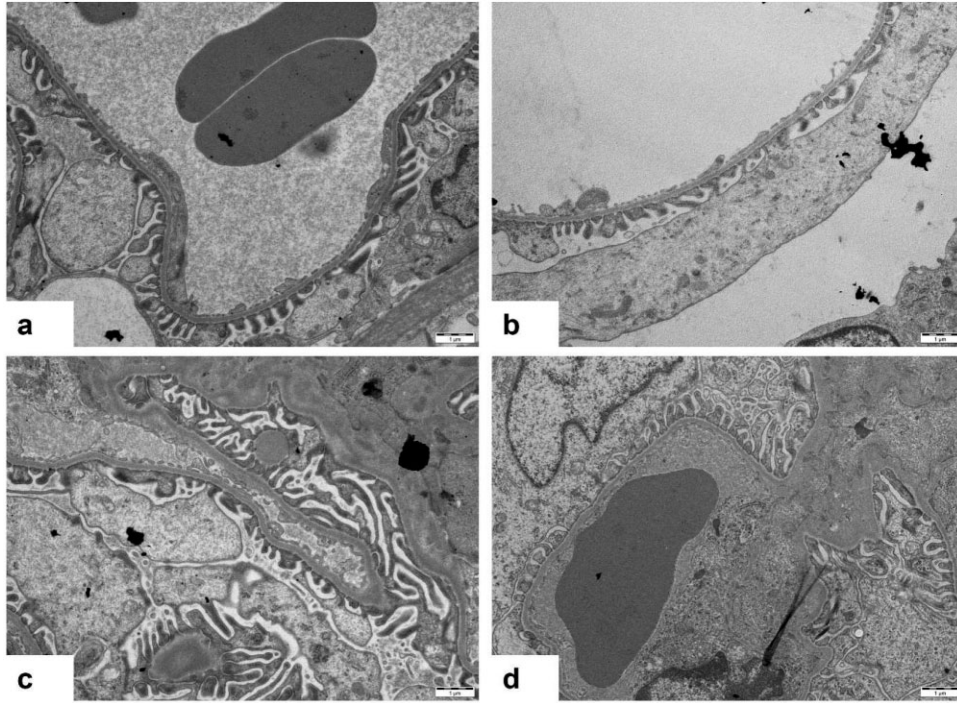
Figure 1: Timeline of *in vivo* experiments.



**Figure 2:** Physical and biochemical analysis of rats. \* $P < 0,05$  vs D, D+2D-CM, D+3D-CM; \*\* $P < 0,05$  vs D; \*\*\* $P > 0,05$  vs D, D+2D-CM, D+3D-CM; # $P < 0,05$  vs post-treatment C; ## $P < 0,05$  vs post-treatment-D.



**Figure 3:** Histopathological and immunohistochemical analysis. \*P<0,05 vs C and D+3D-CM, \*\*P<0,05 vs the other groups; \*\*\*P<0,05 vs C.



**Figure 4:** TEM evaluations. C (a), D (b), D+2D-CM (c) and D+3D-CM (d).

## REFERENCES

1. Isildar, B. *et al.* 2D and 3D cultured human umbilical cord-derived mesenchymal stem cell-conditioned medium has a dual effect in type 1 diabetes model in rats: immunomodulation and beta-cell regeneration. *Inflamm. Regen.* **42**, 55 (2022).
2. Ozkan, S. *et al.* Therapeutic potential of conditioned medium obtained from deferoxamine preconditioned umbilical cord mesenchymal stem cells on diabetic nephropathy model. *Stem Cell Res. Ther.* **13**, 438 (2022).