AN OPTIMIZED ASSAY TO ASSESS MEMBRANE PERMEABILITY CHANGES IN HUMAN GLOMERULAR ENDOTHELIAL CELL LINE

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BACKGROUND

The conditionally immortalized human glomerular endothelial cell line (ciGEnC) resembles morphological features of early-passage primary cells (1). In vivo, they play an important role in filtration under physiological and pathological circumstances. FITC-Dextran assay is widely used to assess endothelial cell monolayer permeability, but it may have many drawbacks. In order to derive a repeatable and representative assay, the baseline permeability of cell monolayer has to be optimized for every cell line. Our aim was to set up an improved FITC-Dextran permeability assay to measure the barrier function of ciGEnC monolayer.

METHODS

CiGEnC is a thermosensitive cell line, proliferates at 33 ℃ and differentiates at 37 ℃. Cells were seeded on 24-well plate Millicell insert membranes and the following factors were tested on the monolayer permeability (n=3/group): 1) the length of proliferation phase; 2) the number of seeded cells; 3) the duration of differentiation. Empty insert containing no cells was used as control (CTL). The changes in permeability were measured using a 40 kD FITC-Dextran assay. We also assessed cell morphology changes under the studied circumstances on standard 24-well plates.

Statistics:

Data are presented as mean±SD. One-way analysis of variance (ANOVA) followed by Tukey post-hoc test was performed in order to test statistical significance.

RESULTS

Factors that influence permeability

Length of both proliferation and differentiation phases:

Increasing the length of proliferation from 1 day to 5 days reduced permeability by 40% as compared to the CTL (Figure 1).

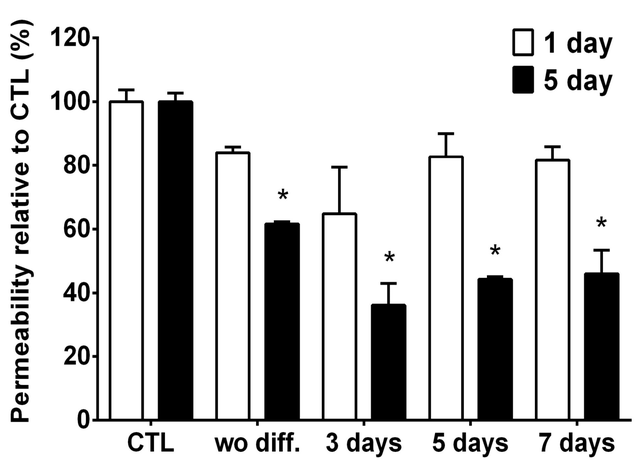

Figure 1. The cells were proliferated and differentiated for the indicated period of time on the insert membranes. Permeability values are expressed as relative to the empty insert. *p<0.05

Cell morphology changed from larger elongated cells (1 day) to reduced cell length and diameter after 5 days of proliferation (Figure 2).

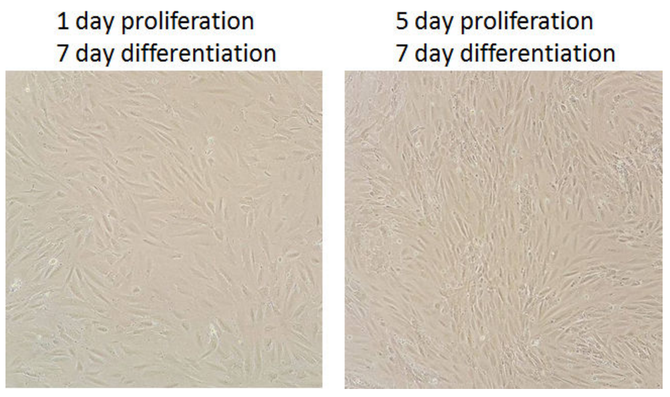

Figure 2. The cells were proliferated for 1 or 5 days and differentiated for 7 days.

Cell number:

With optimizing the number of seeded cells, we reached 88% reduction in permeability compared to empty insert (CTL).

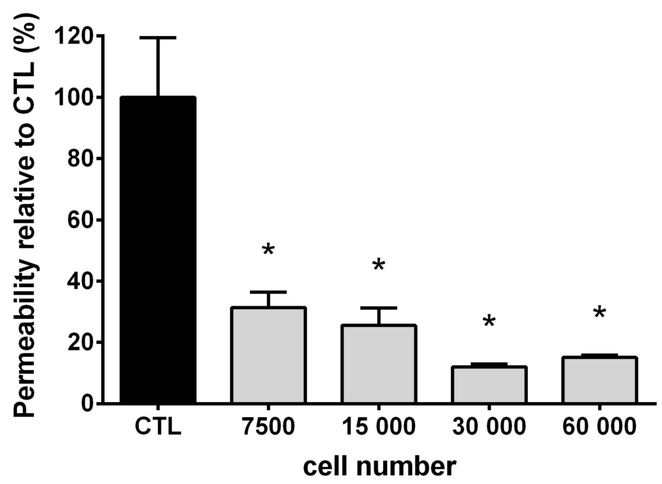

Figure 3. The indicated number of cells were seeded on the insert membranes, and proliferated for 5 days followed by differentiation for 7 days, when permeability was measured. Values are expressed as relative to empty insert. *p<0.05 vs CTL

Different coating methods:

Insert surface coating decreased the cell monolayer's permeability further, reaching a maximum of 94% reduction with fibronectin coating (as compared to CTL).

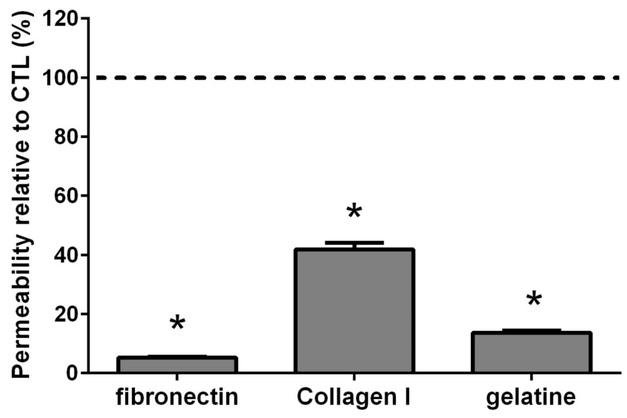

Figure 4. The cells were proliferated for 5 days followed by 7 days of differentiation, when permeability was measured and expressed relative to empty insert (CTL, shown as dashed line). *p<0.05 vs CTL

Assay validation with VEGF

In order to validate the permeability assay, ciGEnC monolayer was treated with vascular endothelial growth factor (VEGF) which is known to increase endothelial cell permeability. VEGF (100ng/ml) increased the monolayer permeability by 19% or 24% after 24 or 48 hours of treatment, respectively.

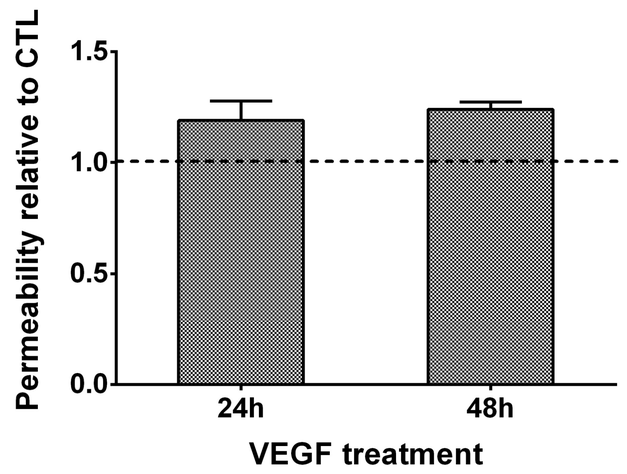

Figure 4. The cells were proliferated and differentiated as described previously. Membrane permeability (relative to CTL) was measured after VEGF treatment for the indicated period.

CONCLUSION

We have established an optimized FITC-Dextran permeability assay for ciGEnC. This assay is eligible to assess the effects of VEGF, other compounds on the membrane permeability of ciGEnC cells.

REFERENCES

1) Kidney Int. 2006 May;69(9):1633-40.

FUNDING

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