COMPARATIVE STUDY OF HUMAN EMBRYONIC STEM CELL SURFACE STRUCTURE USING SEM AND ESEM

Eva Flodrová[a], Vilém Neděla[a], Aleš Hampl[b], Miroslava Sedláčková[b]

[a] Institute of Scientific Instruments of the ASCR, v.v.i, Královopolská 147, 612 64 Brno, Czech Republic
[b] Department of Histology and Embryology, LF MU, Kamenice 5, 625 00 Brno, Czech Republic

Introduction

A widely-used method for microstructural and topographical characterization is scanning electron microscopy (SEM). Using SEM for biological sample observation has specific requirements for its preparation. Especially drying process is critical operation for susceptible samples like human embryonic stem cells (hESCs). The necessity of the sample´s preparation treatments can be eliminated with using environmental scanning electron microscopy (ESEM), but with decreased resolution.

The object of this study was to investigate feasibility of ESEM and SEM for morphological characterization of undifferentiated hESCs cultured on glass substrate.

Colonies of the undifferentiated hECSs were cultured on mouse embryonic fibroblasts in Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno

SEM Observation

Samples were fixed in 2.5 % glutaraldehyde with phosphate buffered saline (PBS), postfixed in 2 % OsO_4 with PBS, dehydrated in ethylalcohol, dried and sputter coated with gold. Due to problems with sample cracking, two methods of drying were applied, critical point drying (CPD) and freeze drying.

Samples were observed with high resolution scanning electron microscope Jeol JSM 6700F with autoemission cathode (accelerated voltage 5 keV, working distance 8 mm).

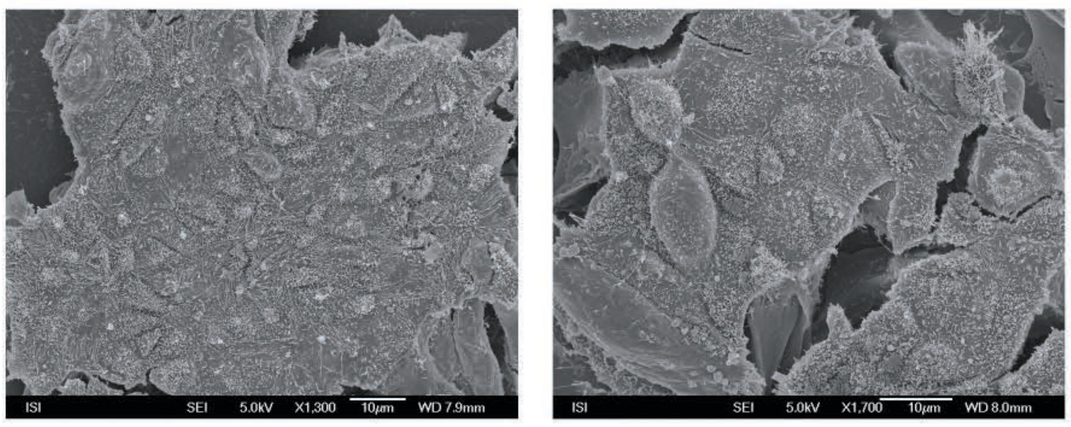

Fig. 5: Samples were dried with CPD method, this predominantly dynamic method involves mechanical stress and cracking of cell colonies, especially in places with higher density. Characteristic of surface morphology has been preserved.

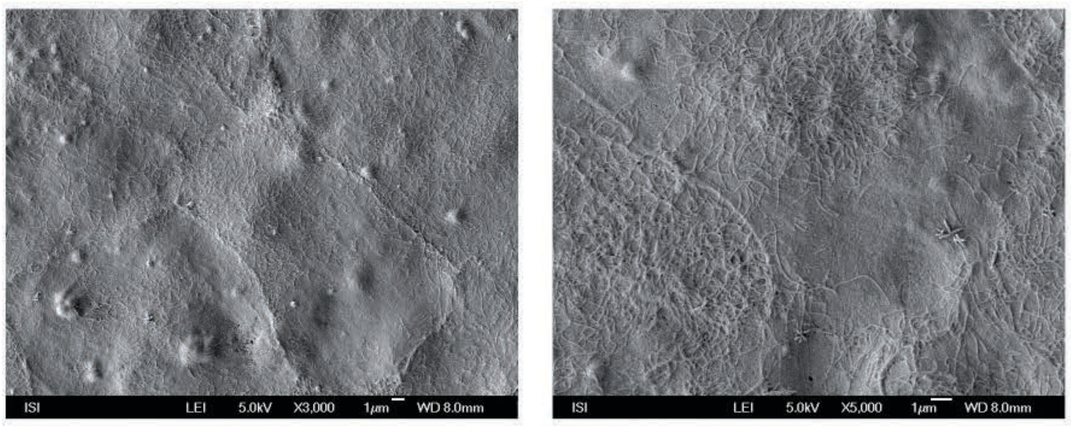

Fig. 6: Samples were dried with a method of freeze drying when the solvent is removed as a vapor by sublimation from frozen sample. The impact of the surface tension on the colony adhered on glass substrate has been kept to a minimum. Results show less shrinkage and structure cracking but with slightly altered surface structure.

Conclusion

The presented results illustrate pros and cons of using SEM and ESEM for microstructural and topographical characterization of hESCs.

Classical SEM allows displaying details of the sample surface in their higher resolution than ESEM. Nevertheless a lot of treatments with inconsistent results are necessary.

The microvilli can be displayed when the appropriate fixation is used, but ESEM ability to display high resolution details of microvilli on the surface of the hESCs is not sufficient. On the other hand ESEM proves ability to display boundaries of the individual cells in the colony of hydrated sample surface which is unrecognizable in SEM.

HESCs imaged with ESEM indicate three-dimensional appearance and with observation in different states of inundation the basic information about altitude differences in colony can be obtained.

For acquiring complex information about hESCs surface the combination of both methods can be recommended.

ESEM Observation

The impact of individual treatment procedures on possibility to ESEM observation was studied. Firstly, the samples were observed in their native state without treatments. Then the fixation in 2.5 % glutaraldehyde with PBS and postfixation in 2 % OsO_4 with PBS were applied

The samples were observed in our experimental ESEM AQUASEM II [4] in a wet mode, Fig. 1 - 2, 4 were taken by ionization detector, Fig 3 is from BSE detector. The glass insert with hESCs was laid on a cooled specimen holder (2°C).

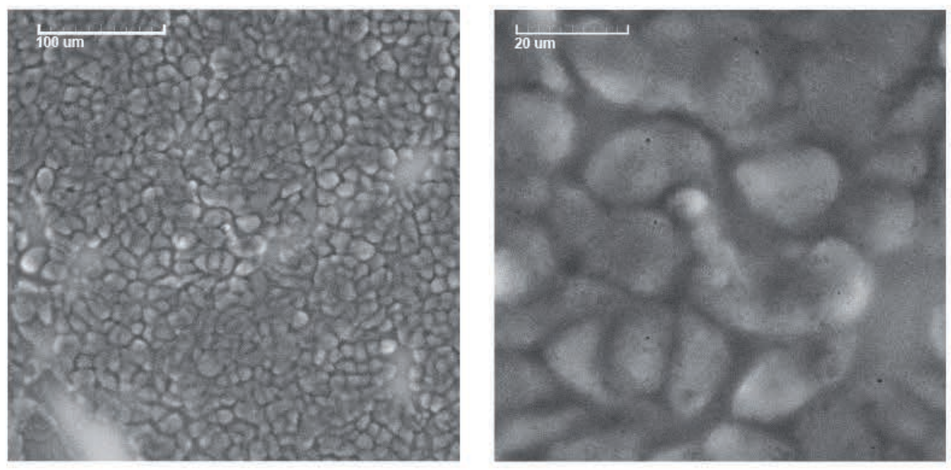

Fig. 1: The colony of hESCs was observed in the native state. Boundaries between individual cells in the colony are well visible and shape variation of the cells can be observed. In consequence of sample instability and degradation processes the sample was covered with mucus which prevented microvilli observation.

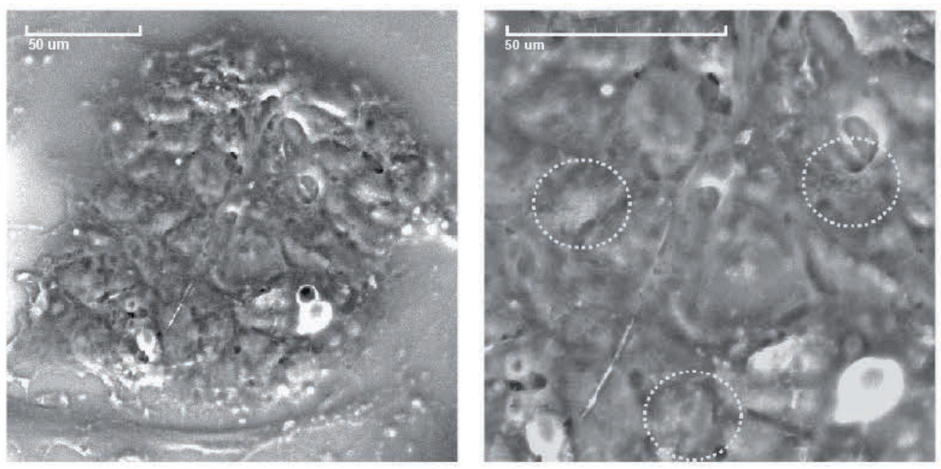

Fig. 2: On the surface of the samples fixed in glutaraldehyde, microvilli are visible (dotted circles). Surface morfology of the individual cell as well as the whole colony can be studied.

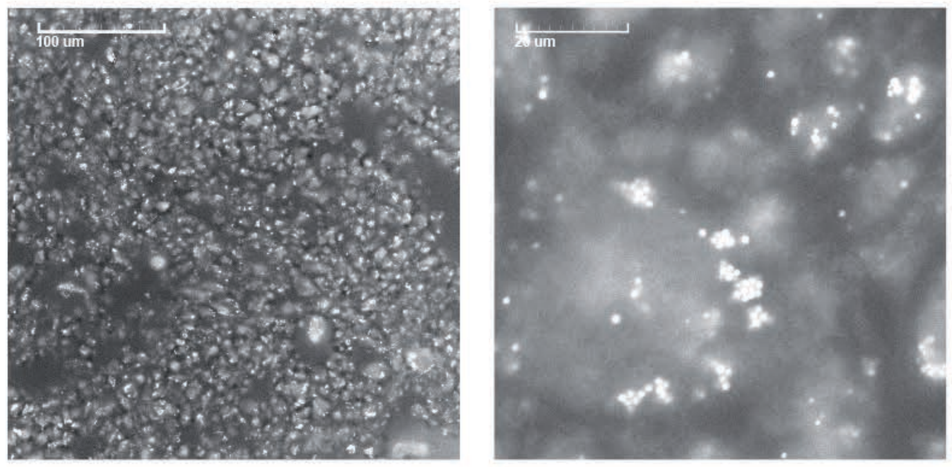

Fig. 3: Inner structure visibility for BSE detector was achieved by using OsO_4 postfixation. The cells boundaries are poorly recognizable, whereas the lipids representing by conglomeration of globules are well visible.

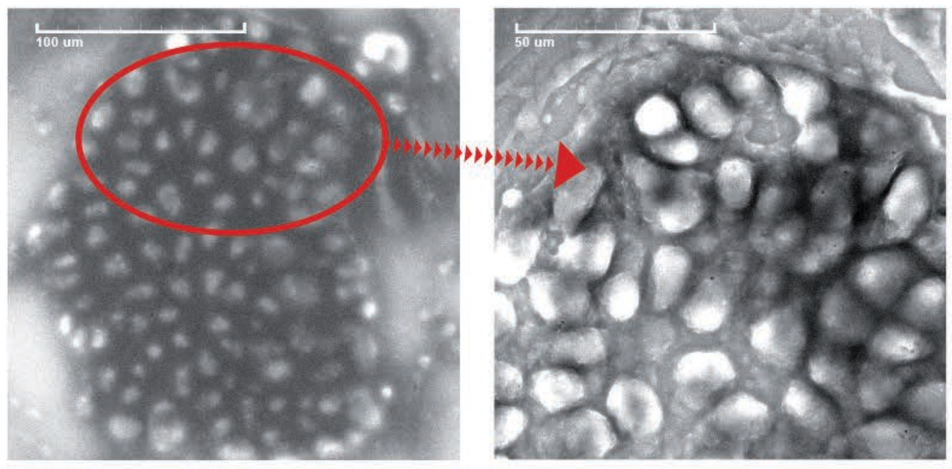

Fig. 4: Partially inundated colony (on the left) can be used for derivation of altitude differences between individual cells.

INVESTMENTS IN EDUCATION DEVELOPMENT

References

[1] Itskovitz-Eldor, J. et al., Mol. Med. 6 (2), 88 (2000).
[2] Mumaw, J.L. et al., Microsc. Microanal. 16, 80 (2010).
[3] Danilatos, G.D., Advances in Electronics and Electron Physics 71, 109 (1988).
[4] Nedela, V., Microscope Res. Tech. 70, 95 (2007).
[5] This work was supported by the Grant Agency of the Czech Republic: grant No. GAP 102/10/1410, by the Ministry of Industry and Trade of the Czech Republic: grant FR/TI1/305 and FR/TI1/118, and by the EU project No. CZ.1.07/2.3.00/20.0103.