SEM and ESEM Observation of Stem Cells

Comparative Study of Human Embryonic Stem Cell Morphology

Studying biological samples with scanning electron microscopy has specific requirements for their preparation. Sample drying is a particularly critical operation for objects such as cultured cells. The requirement for damaging drying step can be eliminated using environmental scanning electron microscopy. This study compares dried and wet samples of cultured human embryonic stem cells. It points to the advantages of both methods and to the complementarity of the information that they provide.

Human Embryonic Stem Cells

Developmental potential of cells of embryoblast in blastocyst stage embryo is tremendous as they produce all cell types of the embryo proper. Cells of embryoblast can be explanted and adapted to in vitro culture thus producing lines of immortal pluripotent cells called embryonic stem cells. The potential of human embryonic stem cells (hESCs) to differentiate into all types of somatic cells in the organism offers a great promise of therapeutic treatments to many incurable diseases. The ability of self-renewal makes hESCs an important research subject of modern medicine and also allows the provision of in vitro human developmental model that provides a unique opportunity for investigating key factors that are impossible to study in vivo [1].

An increasing number of publications are focused on investigating properties of in vitro propagated hESC using various microscopic approaches with the aim to characterize pristine undifferentiated hESC and the changes associated with entry of hESC into differentiation pathway. Still, a surface microstructure has not yet been studied in detail. Many hESCs contain extensive specific cell structures called microvilli whose role is poorly understand. In general, microvilli play an important role in metabolic regulation, calcium signaling, and the establishment of polarity in embryonic epithelium; however, there have been no documented reports, to our knowledge, of cell-cell signaling or migration cue transmission between cells through microvilli in hESC [2].
Widely used method for visualization of cells and biological tissues is scanning electron microscopy (SEM).

For SEM analysis the samples require multiple processing steps that include stabilization (fixation), dehydration and prevention from charging. Therefore, imaging of cells and tissues using environmental scanning electron microscopy (ESEM) may provide alternative approach that is void of charging artifacts thanks to a minimum of treatments. This method allows the observation of samples without prior dehydration and minimizes or eliminates the need for the sample preparation treatments described above. Cooled samples are placed in a high pressure environment of gas or water vapor which is ensured by using a series of pressure-limiting apertures and a differentially pumping chamber [3, 4]. Appropriate conditions in the specimen chamber can be obtained by adjusting the chamber pressure and temperature of the cooling stage.

**Materials and Methods**

For SEM cultured hESC were classically fixed in 2.5 % glutaraldehyde in 0.2 M phosphate buffer (PBS) pH 7.1, postfixed with 2 % OsO$_4$ in PBS, dehydrated in ethanol, dried and conductive covered with gold layer for SEM observation. 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.
ESEM observation allows minimizing treatments; a necessary amount of treatment steps and its impact on possibility of observation was studied. Firstly, samples on glass support substrate were observed in their native state without treatments. Then the fixation with 2.5 % glutaraldehyde in PBS and postfixation with 2 % OsO$_4$ in PBS were applied. Samples were observed in our experimental ESEM Aquasem II [4] in a wet mode.

**Scanning Electron Microscopy Observation**

Firstly, samples were dried with CPD method. This predominantly dynamic method involves mechanical stress and cracking of hESC colonies, especially in places with higher cell density. However, characteristics of surface morphology were preserved (fig. 1A). The cell surfaces appear embossed, some cells show bulging relief. Numerous microvilli extend from the cell surfaces; their structure and arrangement are well preserved. Alternative drying with method based on freeze drying principle contains in quickly frozen and solvent sublimation from sample. The impact of surface tension on colony adhered on glass substrate has been kept to a minimum (fig. 1B). Results show much less shrinkage and structure cracking but with slightly altered surface structure. The cell surface is flat and microvilli do not extend from cell surface, they are lying. Relief of cell surface seems to be somewhat inconspicuous.

**Environmental Scanning Electron Microscopy Study**

The first ESEM observation of hESCs colony was carried out in their native state (fig. 2A). Real size and shape of cells are preserved, and 3D configuration remains unchanged. Boundaries between individual cells in colonies are well visible and shape variation of cells can be observed. In consequence of sample instability and degradation processes, the sample was covered with a film impeding microvilli observation.

Secondly, the samples were stabilized with basic glutaraldehyde fixation. The results are visible in figure 2B, cell boundaries are well recognized as well as some details of surface morphology (microvilli and their arrangement on the surface of individual cells) (dotted circles).
Lastly, the double fixation method using glutaraldehyde and osmium tetroxide was applied. In this case, not only surface morphology can be observed, but also some internal structures become visible due to their affinity to osmium. This heavy metal is bound to lipid components and thus enables their visibility for BSE detector (fig. 2C). Using BSE detector, cell boundaries are badly recognizable; however, the lipids represented by conglomeration of globules are well visible.

**Conclusion**
The characteristics of surface morphology of cultured hESCs obtained here by using different types of microscopes and preparation methods mirror their specific advantages and disadvantages. Classical SEM allows displaying details of the sample surface in their higher resolution than ESEM nevertheless many treatments are necessary.

The advantage of ESEM lies in the potency to study cells in situ without artificial changes caused by cell treatment. The ability to display high resolution details of microvilli on the minimally treated and fully hydrated sample surface of the hESC colonies is not sufficient yet in our ESEM. Still, boundaries of the individual hESC cell in the colony of hydrated sample surface are well visible, while in SEM are poorly recognizable.

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**References**

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