POSSIBILITIES OF USING ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY TO STUDY OF HUMAN EMBRYONIC STEM CELL

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Introduction

The potential of human embryonic stem cells (hECS) to differentiate into all types of the cells in the organism and cell populations derived from these pulipotent cells offers promise of therapeutic treatments to many incurable diseases. A surface microstructure has not yet been described in detail. Many (hECS) contain extensive specific cell structure called microvilli whose role is poorly understood.

The object of this study was to investigate the possibility of using environmental scanning electron microscopy (ESEM) for characterization morphological specifics on an undifferentiated hESC colony surface cultured on glass substrate.

Materials and methods

In this project colonies of undifferentiated hECSs grow on the supporting layer of primary human embryonic fibroblasts (Department of Biology, Faculty of Medicine, Masaryk University) were observed. The samples were fixed by 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 2% osmium tetroxide and rinsed with 0.1M phosphate buffer.

Observations were made with experimental environmental SEM AQUASEM II using Ionization and YAG BSE detectors. The water wapour flow in the specimen chamber and temperature of the sample was regulated to a relative humidity from 100 to 80 %.

The samples of hESC colonies on the glass substrate were placed on a Peltier cooled specimen holder and covered by a drop of PBS buffer. Consequently the liquid was slowly evaporated from the sample.

Results

The results obtained by using different types of microscopes show their specific advantages and disadvantages. Classical SEM allows to display details of the sample surface in higher magnification and resolution than ESEM (Fig. 1) nevertheless a lot of treatments are necessary.

ESEM ability to display high resolution details of microvilli on the surface of the colonies is not sufficient due to decreasing of amount of the low energy SEs passing through thin water layer covering the sample surface to the detector. On the other hand, the individual hESC cells in the colony of hydrated sample surface are well visible (Fig. 2), better than in fully treated surface displayed by SEM. Slight indications of microvilli are visible on long-term cultured samples with high specific cell structures density (Fig. 3, markers). In view of the fact that human embryonic stem cells are very sensitive to change of environment, observation of full untreated native samples is not yet feasible. Therefore, at least the chemical fixation is needed for sample stabilization.

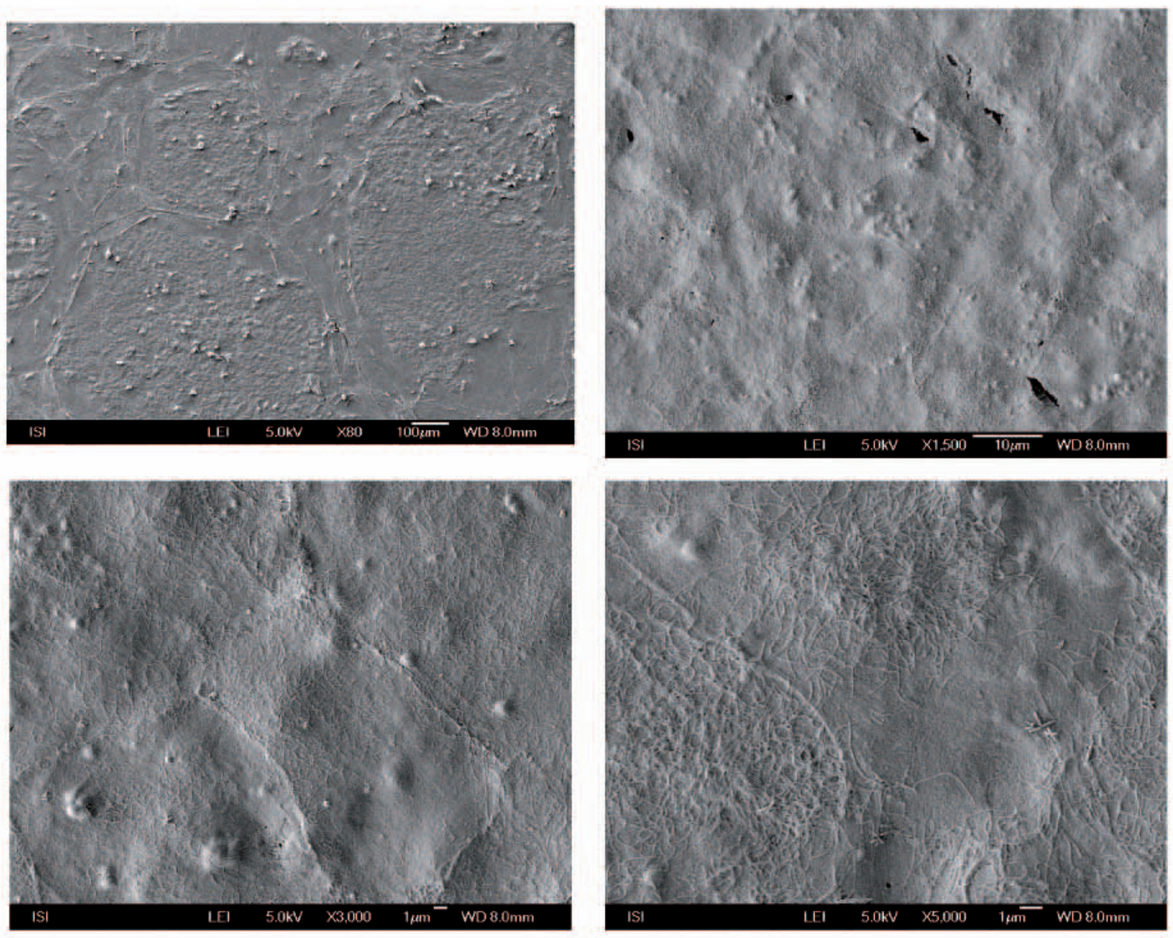


Fig. 1: Colonies of hESC fully treated for SEM (fixed, postfixed, dehydrated, dried and covered with gold layer)

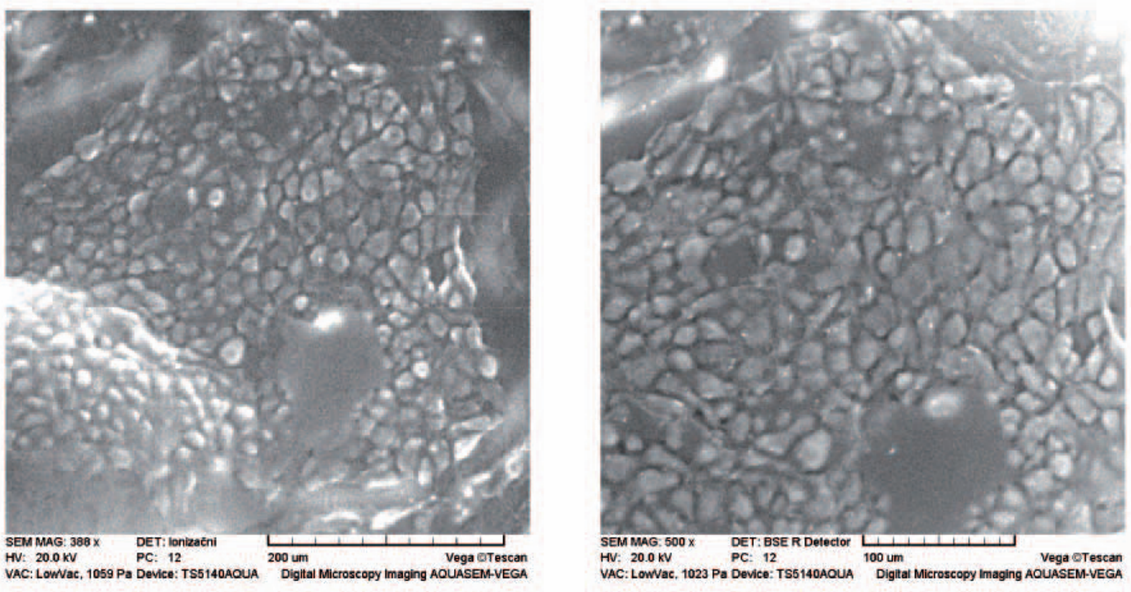


Fig. 2: The fully hydrated colonies of hESC observed with environmental SEM AQUASEM II. Boundaries between the hESC cells in colony are well visible.

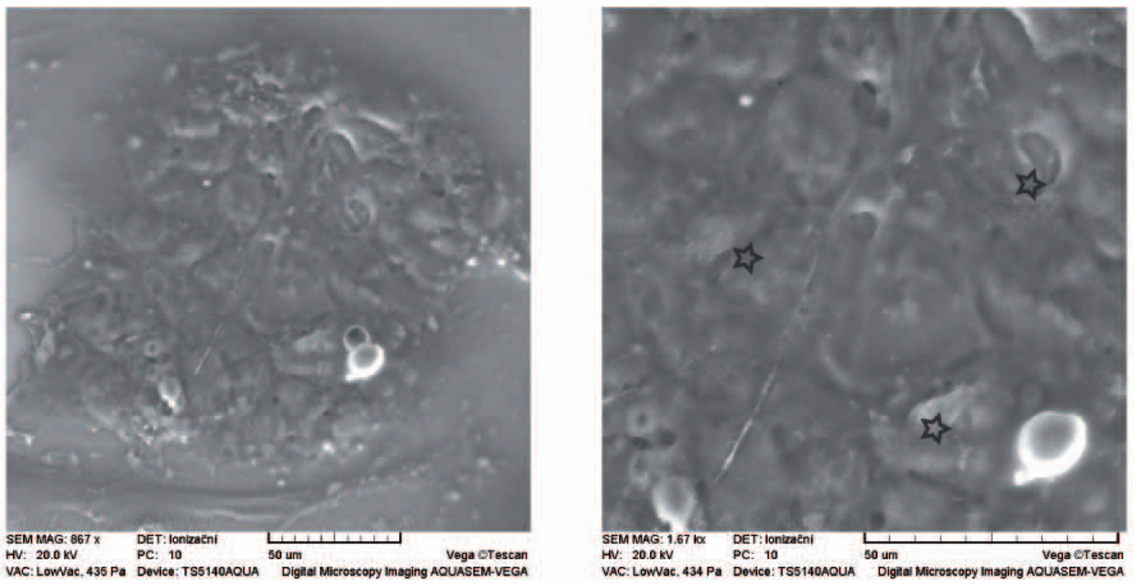
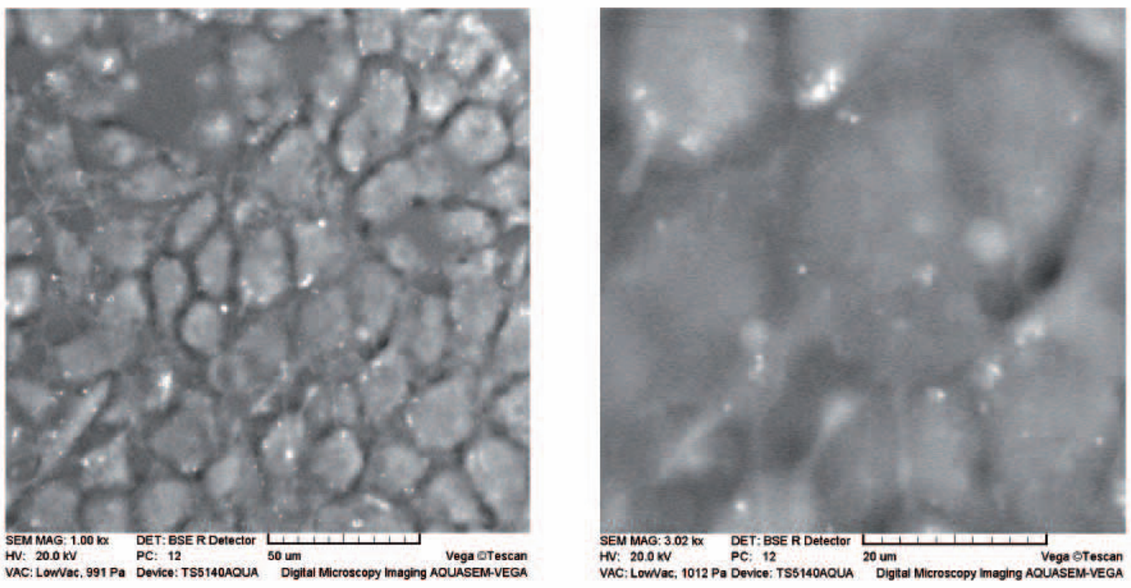


Fig. 3: Long term cultured colony of hESC dense coverec by microvilli. Slight indications of microvilli are pointed out by markers.



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