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A study of the applicability of xenon clathrates for the preservation of human stem cells and skin fragments

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Rapid development of regenerative medicine calls for new approaches to the problem of long-term preservation of cells, tissues and organs. This is one of the key issues in, e.g., transplantology and tissue engineering. The existing cryopreservation techniques are largely limited by the toxic effects that commonly used cryoprotector components such as DMSO [5] and glycerol [4] exhibit on the stored bio-objects [1] and eventual recipients [2]. Xenon gas can be a viable alternative to DMSO due to its ability to form clathrates in aqueous solutions, which effectively prevent the formation of ice crystals and therefore limit the damage to the cells [3]. Due to its low toxicity, xenon gas is widely used nowadays in anesthesia as well as a medicine for treatment of various diseases [6]. Several earlier studies have demonstrated the possibility to use xenon for cryopreservation of cell suspensions [7]. In another work, a mixture of xenon and oxygen at a pressure of 6 to 7 atm has been used for short-term cryopreservation of mammalian tissues [8]. However, to the authors' knowledge, all previous works have studied either cell suspensions or animal tissues, while very limited information has been collected on human tissues. Moreover, no quantitative data on the properties of clathrates used for conservation and characteristics of the preserved cells (e.g. viability) have been provided. The purpose of this study is to explore the possibility of using xenon clathrates as a cryopreservation agent for biological objects of human origin, such as human stem cells and skin tissues.

A custom-designed sealed chamber equipped with pressure and temperature sensors to monitor the parameters of clathrate formation and a window to visually register the clathrate point in the system was built for the experiments. Human stem cells and skin samples were preserved in clathrates for 1 and 2 days, respectively. The damaging effect of xenon clathrates on the stages of clathrate formation and storage was evaluated using an environmental scanning electron microscope (Zeiss Evo) equipped with a cryostage. The viability of the skin samples after cryopreservation in xenon clathrates was evaluated by expansion of fibroblasts in DMEM with 10% fetal bovine serum [9]. Histology of the clathrates-preserved and reference (intact) skin samples was performed using standard techniques [10]

The studies showed no visible damaging effect of xenon clathrates on stem cells during clathrate preservation and storage (Fig. 1). However, the cells were almost completely destroyed during the re-activation stage. On the hand, the skin samples preserved in xenon clathrates did not exhibit any histological difference from the reference specimens. The skin samples stored in xenon clathrates exhibited a steady output of fibroblasts with positive dynamics and did not differ from the reference specimens after 25 days of culture (Fig. 2).

In conclusion, our preliminary studies demonstrated that xenon clathrates can be used as a preservation agent for storage of human skin. Preservation, storage and re-activation stages exhibited virtually no impact on the skin samples. At the same time, while the stem cells were seemingly intact at the time of preservation and storage, they were almost completely destroyed during re-activation. This might indicate that the suspension of stem cells is less resistant to decompression as compared to skin samples and requires some adjustment in our procedure. In the future work we plan to address this issue, evaluate the percentage of damaged cells in the

current and future cryopreservation protocols and make a detailed description of the histological skin fragments preserved in xenon clathrates.

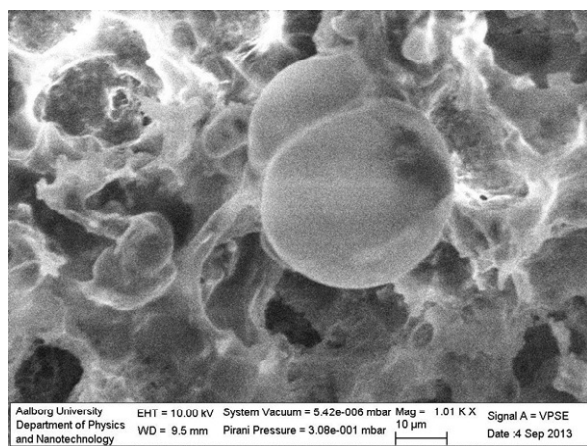


Fig. 1. Stem cells preserved in xenon clathrates. Note the intact cell membrane, no ice crystals present.



Fig. 2. The expansion of the fibroblasts of the skin sample after preservation in Xe clathrates. Tendency to form a monolayer on the 25th day of cultivation was observed

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