

Abstract: Cryopreservation of cells is a complex process with many useful applications in basic biological research, medicine and agriculture. In this work we deepened the current understanding of the cryopreservation process both at physical and biological level. Results include characteristics of selected cryoprotectants (primarily DMSO, trehalose, antifreeze protein ApAFP752) in liquid phase, during phase transition and in solid phase, as well as their impact on cryopreserved cells states. Specifically, the level of cell viability, state of cell membrane and condition of cell nucleus (nuclear membrane, chromatin condensation, DNA strand breaks) are monitored over several time points after thawing. It is shown that S-phase cells (NHDF and MCF7 lines) suffer massive collapse of replication forks during cryopreservation which makes them much less suitable for cryopreservation than cells in other phases of the cell cycle. Several methods (most importantly Atomic Force Microscopy, Confocal Fluorescence Microscopy and Flow Cytometry) were used to examine the post-thaw state of cryopreserved cells. The acquired insights into cryodamage of cells can lead to optimization of current cryopreservation protocols and to more thorough evaluation of efficacy of future novel cryoprotectants.