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Cryopreservation of living cells using electron microscopy fixation methods

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Rapid cooling to minimize ice crystal growth can be applied for cryopreservation as well as sample preparation for (electron) microscopy. However, the methods used in these two fields are very different. To preserve the structure of living samples for electron microscopy in a close to native state, they have to be completely vitrified with minimal use of cryoprotective agents. We found that complete vitrification is not necessary for successful cryopreservation of mammalian cells. However, ice crystal size (not number or total amount of ice) has to be minimized. Therefore, sample preparation methods for (electron) microscopy could also help improving the outcome of cryopreservation. We found that standard preparation methods are not very suitable for cryopreservation, likely because rapid warming is not possible with these methods. However, we found that the recently developed method of self-pressurized rapid freezing is very suitable for cryopreservation. Sealed metal tubes with high thermal diffusivity containing the samples are plunged into liquid cryogen. Internal pressure builds up reducing ice crystal formation and therefore supports reversible cryopreservation through vitrification of cells. After rapid rewarming of pressurized samples, viability rates of >90% can be reached, using human cells (HeLa). This is comparable to best-performing of the established rapid cooling devices tested. In addition, the small SPRF tubes allow for space-saving sample storage and the sealed containers prevent contamination from or into the cryogen during freezing, storage, or thawing.

Biography

Jan Huebinger has his expertise in the development of cryo-immobilization techniques of living samples. He co-developed and evaluated new methods for cryo-fixation for cryo-electron microscopy and for cryopreservation of living cells. More recently he has co-developed a method that allows to reversibly cryo-arrest living cells on a light microscope. This allows to image highly dynamic processes in living cells with methods that necessitate long acquisition times, e.g., super resolution microscopy, at multiple points in time.

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