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Project

Optimized cryopreservation of primary human hepatocytes for pharmacological and toxicological research

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Optimized cryopreservation of primary human hepatocytes for pharmacological and toxicological research

Adherent cultures of primary hepatocytes are a valuable tool for various kinds of pharmacological and toxicological studies, since they play the most important role in the metabolism of drugs and toxins. An induction of drug metabolizing enzymes is usually already detectable in cell culture experiments, rendering further animal testing unnecessary.

Primary human hepatocytes are obtained from the tumor-free margin of tissue removed during liver surgery. The availability of such tissue is therefore very sporadic. From one gram of liver tissue, more than 15x106 viable hepatoytes can be isolated, which is often more than can be processed in a single set of experiments. Therefore, it is necessary to find appropriate long- and medium-term storage conditions in order to fully exploit this cell source.

Cells and tissues are either stored at 4°C or frozen. Since cold-induced cell injury increases with time, cold storage is limited to a few hours or days. For cryopreservation, in contrast, the crucial procedures of freezing and thawing affect cell quality, while prolongation of storage time has virtually no effect. Frozen cells can easily be shipped to partner laboratories, enabling an extended use of cells even without direct access to the tissue or without established isolation procedures.

However, hepatocytes are difficult to cryopreserve (Fig. 1) and very sensible towards freezing/thawing. Furthermore, they lose their attachment ability after thawing, a fact that makes thawed hepatocytes useless for conventional cell culture.

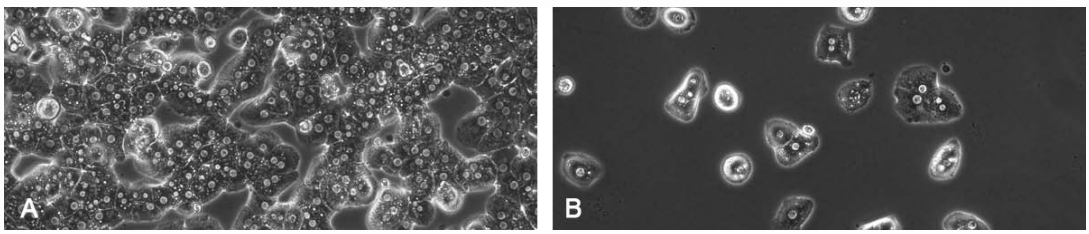

Fig. 1: Adherent cell culture of freshly isolated primary rat hepatocytes 24 h after seeding (A) and after freezing in cell culture medium with 10% DMSO, thawing and 24 h of cell culture (B). The initial number of viable cells was identical in both samples. Non-adherent cells were washed off after adherence phase.

Cryopreservation injury is currently explained primarily by physical processes: Fast freezing leads to intracellular ice crystal formation and rupture of the cell membrane. Slow freezing leads to extracellular ice formation and subsequent hyperosmotic stress (Fig. 2). In both cases, cell death after thawing occurs. However, even if hepatocytes are not lethally damaged after thawing, a large proportion of them fail to attach to culture surfaces or die at a later time point. Recent findings, including own results, suggest that in addition other, cell biological processes may play a crucial role and might be influenced by the composition of the cryopreservation medium.

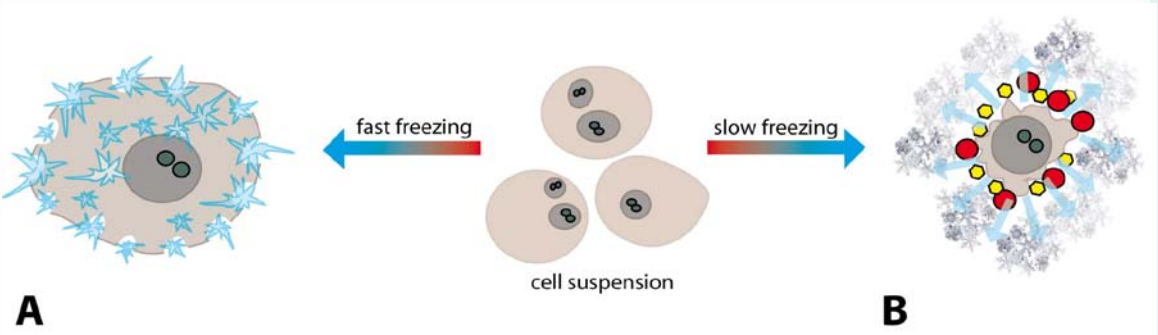

Fig. 2: Cell injury during cryopreservation (adapted from P. Mazur, 1972). A: Fast freezing results in intracellular ice crystal formation and rupture of the cell membrane. B: Slow freezing leads to extracellular ice formation, increase of extracellular solute concentration and subsequently to osmotic stress.

Goal of our project is – based on previous experience with cold storage of various cell types and cryopreservation of rat hepatocytes and porcine aortic endothelial cells – to optimize cryopreservation of primary human hepatocytes. By improving cell quality after freezing/thawing, a wider use of human hepatocytes for pharmacological and toxicological research is to be expected, thus reducing the number of animal experiments.

Project Manager

Prof. Dr. Ursula Rauen

Study of medicine in Düsseldorf and in Aberdeen, Scotland, 1984-1991. Internship 1991/92 (Tübingen), MD 1993 (Düsseldorf). 1993-2008 Research assistant at the Institute of Physiological Chemistry, University Hospital Essen. Habilitation (Physiological Chemistry) 2000. Since 2008 Professor of Physiological Chemistry, Medical Faculty of the University Duisburg-Essen. Research: Mechanisms of cold-induced cell and tissue injury and of preservation injury, development of preservation methods.

Research Team

Dr. Gesine Pless-Petig

Studies in biology at RWTH Aachen 1994 - 2000; diploma in 2000. 2000-2007 PhD student and associate researcher at the group of Experimental Surgery, Charitè - Universitätsmedizin Berlin. Graduation in 2007, thesis on “Use of rejected donor organs as cell source for extracorporeal liver assist devices”. Since 2008 postdoc at the Institute for Physiological Chemistry, University Hospital Essen; research on cold storage and cryopreservation of hepatocytes.

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