Supercooling Storage for the Transplantable Sources From the Rat and the Rabbit: A Preliminary Report

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ABSTRACT

Background. It is necessary to store the transplantable sources for a certain period during the variety of manipulation processing steps. The method used to preserve (depending on conditions of solvent, temperature, periods, density, and physical impulse, etc.) can affect the safety and efficacy of the samples. Supercooling refers to a phenomenon of lowering the temperature below its freezing point without freezing. We investigated the possibility of supercooling for the preservation of cells and organs according to the limited conditions.

Method. The viability of mesenchymal stem cells (MSCs) derived from the intra-abdominal fat of the New Zealand white rabbit were observed, and the neonatal rat kidneys were maintained in histidine-tryptophan-ketoglutarate solution and stored at various temperatures for 48 hours. The supercooling refrigerator was used for −2 °C and −5 °C in controlled preservation conditions. We observed and compared histopathological changes of samples at each temperature condition.

Results. As time passed, the number of rabbit MSCs decreased in each group with storage temperature. At room temperature, the number of viable MSCs decreased rapidly, but the number of MSCs tended to decrease slowly in the cooling and supercooling groups. The rat kidneys preserved on supercooling temperature at −2 °C tended to have the least damage on the cortex and medulla parenchyma.

Conclusions. The difference in damage of transplantable sources by storage temperature conditions is the evidence that effectiveness may depend on the storage method. It is necessary to determine further optimal supercooling temperature of the preservation methods with various cells, tissues, and organs in the future.

LONG-TERM preservation of biological materials such as cells, tissues, and organs accompanying the development of regenerative medicine, stem cell therapy, and transplantation technologies has been represented by many researchers as an under-resourced, disregarded, or even careless field. Protracted preservation of organs and other complicated tissues is an exceptionally integrative process and often even incremental breakthrough requires associating knowledge from mechanical engineering, cell biology, tissue engineering, structural biology, and many more fields.

The cell, tissue, and organ specimens isolated from the donor cannot be used immediately after isolation; therefore, there is a demand for storage under suitable conditions [1,2]. Currently, the extended application of organ transplantation or cell therapy, a great advance in the modern

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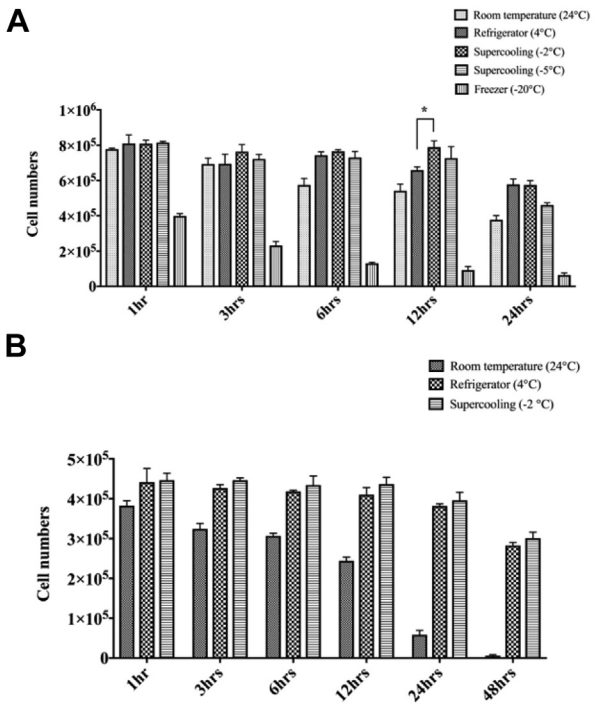

Fig 1. Rabbit adipose-derived mesenchymal stem cells viability in various temperature control conditions. (**A**) The number of mesenchymal stem cells (MSCs) in Hartman solution during 24-hour preservation. (**B**) The numbers of MSCs in normal saline solution during 48-hour preservation.

medical practice, can be hampered by the shortage of donors [3] and the lack of optimal preservation methods for isolated organ or cells. The significant objective of organ preservation for transplantation is to implement a well-maintained organ graft that will perform primary regular functions for a receiver for their lifetime. Although the lack of procedures for balanced preservation of isolated organs is hampering the expansive value of organ transplantation in the hospital settings presently, a number of new procedures have been examined and announced to admit long-term organ preservation [4]. However, there are numerous technical and functional challenges for the successful preservation of human organs that at the outset would seem to be impossible based on the existing circumstances of low-temperature science. Many prominent multidisciplinary fields perform typical preservation of different types of cells; however, the intricacy of tissues and organs introduce many new complications that have demanded ingenious approaches and technologies to be examined [5]. Ice crystallization for the preservation of tissue and organ becomes much more obscure and onerous to balance through the application of conventional cooling systems and cryoprotectants. The management or prevention of intracellular ice formation, one of the eminent reasons for cell destruction [6] or extracellular ice formation, is imperative for the successful preservation. Vitrification procedures for the prevention of ice in tissues that comprise muscle pieces, cartilage discs, and short lengths of blood vessels were examined [7]. However, use of these procedures in tissues and organs is restricted by cryoprotectant diffusion and cytotoxicity.

Supercooling refers to a phenomenon which is the process of lowering the temperature below its freezing point without freezing. In this strategy, the supercooled state is achieved when the refrigerating compartment is capable of provoking an unfrozen status below the freezing point readily and rigidly by using an electromagnetic field [8]. Therefore, in this present study, we sought a supercooling preservation method suitable for cells, tissue, and organ for an extended period. Supercooling prevents water crystallization and maintains cells at a low temperature under isotonic status.

Several preservation techniques such as freezing, cooling, fixing, drying, adding antifreezing materials in subzero temperatures, and cryopreservation using dimethyl sulfoxide and other compounds usually conclude in low feature tissues as a result of cellular lysis and further degradation [9]. Superior viability and functionality of primary hepatocytes and whole liver [10–13], lungs [9] human ovarian cortex [14], and yeast cells [15,16] can be achieved by this customized supercooling. Although the precise mechanism of supercooling preservation is still unknown, this newly invented preservation method may provide breakthroughs in a diversity of scientific and industrial technologies.

This study explored the feasibility of supercooling as a preliminary method with cells and organs according to the controlled conditions.

MATERIALS AND METHODS

Animal Experiment

All the procedures were conducted with the approval of the ethics committee of Konkuk University in accordance with the Institutional Animal Care and Use Committee (IACUC) guide for the care and use of the laboratory animals (KU16202).

Isolation and Culture of Adipose Tissue–derived Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) derived from the intra-abdominal fat of the New Zealand white rabbit (1 kg, male) (Samtako Bio, Osan, Korea) using 0.1% (w/v) Collagenase, Type 1 (Worthington Biochemical, Lakewood, New Jersey) was digested for 30 minutes. Next, the primary cells from adipose tissue stromal vascular fraction were seeded at a 5×10^4 cells/cm^2 using DMEM/F12 (Gibco, Gaithersburg, Maryland) containing 10% fetal bovine serum (HyClone, GE Lifesciences, Pittsburgh, Pennsylvania) and 1% antibiotic-antimycotic (Gibco) and then incubated at 37 °C in 5% CO_2. After reaching 80% ~ 90% confluency, the cells were subcultured to the next passage.

Preservation of MSCs

Single cells using trypsin/ethylenediaminetetraacetic acid (Gibco) were prepared and suspended in Hartman's solution (Daihan Scientific, Gangwon-do, Korea) with 1×10^6 cells/mL and sterile