Suspended Animation

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Date: 8/26/05
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Abstract:

Suspended animation is a state in which all the cell division and movement are stopped. While many organisms can arrest their life processes, it is not known whether the human body can enter such a state. Placing a human body or organ in suspended animation would be of great importance for human life preservation and organ transplantation. In this project, we tested the possibility of preserving human lymphocytes and stem cells at low temperature. Cell freezing was performed in strictly controlled conditions. Cell viability and protein expression before and after freezing were tested using flow cytometry, a technique that allows the rapid analysis of a high number of cells. Our results showed that cell viability and expression of cell surface receptors was not significantly affected by freezing. Thus, we conclude that human lymphocytes and stem cells can be stored at low temperatures, and then used for research or clinical purposes. The freezing conditions used for preserving cells can be tested in the future for the preservation of human organs or tissues.

Introduction:

Suspended animation is similar to hibernation, in which all life processes stop and enter a state of pause. During suspended animation, metabolism, cellular activity, body temperature, respiration and heartbeat decrease, the oxygen is cut off from the body, and the cells stop to divide yet they are still alive. Interestingly, the body will be preserved with no deterioration and it will be protected from injuries. Some organisms like ground

squirrels and many other mammals could enter the state of suspended animation at certain times, such as during cold winters. Recent studies have shown that mice could be put in a state of suspended animation by changing them from warm-blooded to cold-blooded animals. Could this be possible for humans? Several cases of people that survived in extremely cold conditions have been reported. However, many more have died upon exposure to extreme cold for similar periods of time.

Inducing a state of suspended animation in humans can be very useful; the doctors can put the patients with serious injuries into a “stop” to avoid deterioration of their tissues, while they fix their injuries. Also, placing human organs or tissues in such a state would have an enormous impact on transplantation since organs such as the heart or lungs can survive outside the body for only up to six hours.

The first step towards achieving suspended animation of the human body or tissues is to preserve human cells. In this study I have investigated the possibility of preserving human white blood cells, namely lymphocytes, by freezing them in controlled conditions. For this, I have used human lymphocytes isolated from fresh blood and tested their viability and expression of cell surface receptors prior to freezing and after thawing. A successful preservation of the cells can be considered as the basis for achieving suspended animation of whole bodies in the future.

Procedures:

Summary

1) Separate the lymphocytes from blood samples
2) Take some lymphocytes and count them under a microscope.

3) Freeze half of the cells
4) Viability testing by flow cytometry of fresh and defrosted cells
5) Cell surface protein analysis by flow cytometry of fresh and defrosted cells

Separation of lymphocytes

1) Dilute blood with same volume of RPMI (1 to 1 ratio)
2) Add 10 ml of diluted blood to 5ml Lymphocyte Separation Medium (be very gentle when adding blood to the Lymphocyte Separation Median, make sure the blood stays on the top)
3) Spin down for 30 minutes at 3000 RPM with no brake in the centrifuge
4) Take the second layer (lymphocyte) out of the tube
5) Add 15 ml RPMI to wash the lymphocyte
6) Spin down for 10 minute at 2000 RPM and 3 brake
7) Dump the supernatant and collect the pellet on the bottom
8) Count cells using a microscope

Procedure for freezing cells

1) Spin down lymphocytes which are suspended in culture medium for 10min at 1800 RPM.
2) Discard the culture medium
3) Add 1 ml of freezing solution containing fetal calf serum and dimethylsulfoxide (DMSO) to the cell pellet
4) Re-suspend the cells in freezing solution

5) Transfer the cells into 1 ml freezing tubes and immediately place the tubes on ice.

6) After 10 minutes, place the freezing tubes in a -80^{o}C freezer for overnight storage.

Procedure for defrosting cells

1) Take the freezing cells and put them into 37 degrees Celsius water.
2) Transfer the cells into a bigger tube
3) Add 10 ml of RPMI
4) Spin down for 10 minutes with 1000 RPM.

Staining

1) Add 20 microliter of fluorescent antibody to 250,000 cells
2) Vortex
3) Incubate for 20 minutes at room temperature
4) Wash cells, re-suspend in running buffer
5) Run on the flow cytometer using Multi Set

Viability Test

1) Add 10 ul of 7-AAD (7-amino-actinomycin D) to 50 ul of the sample
2) Incubate for 10 minute
3) Use 1 ml of lysing solution (R&D)
4) Spin down for 5 minutes
5) Discard supernatant and vortex cells
6) Add fixing solution

Material:

1) Human Blood Samples. Three blood samples obtained from healthy volunteers were used in this study.

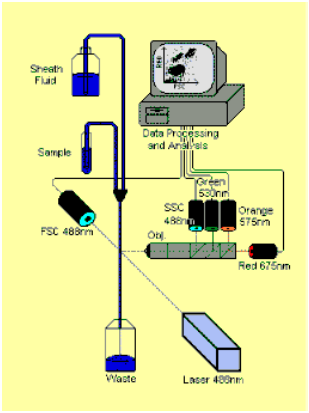
2) Antibodies. Fluorescent antibodies that react with cell surface proteins expressed by lymphocytes were used for cell staining. The following antibodies were used: anti-human CD19, CD3, CD4, CD8, CD45, and CD16/56.

3) Lymphocyte separation medium. A solution separates the blood samples into different layers (according to the density of the cells in the blood) by spin down in the centrifuge.

4) Centrifuge. Use for wash and separate the lymphocytes.

5) Flow Cytometry (diagram of flow cytometer below). First, the sample is taken by a buffer solution called sheath fluid through the laser beam. Some of the laser energy is absorbed by the cells, and then emitted as fluorescence signals. These signals are captured by several photo-multipliers, and further send to a powerful computer. Upon processing of these signals, the computer provides the researcher with information

regarding the number, the size, and the appearance of the cells, as well as the presence of certain cellular proteins on the cells surface. The sheath fluid then takes the cells to a waste container.



6) Vortex. Use for mixing antibody with lymphocytes.

7) Microscope. Use for cell count.

Results:

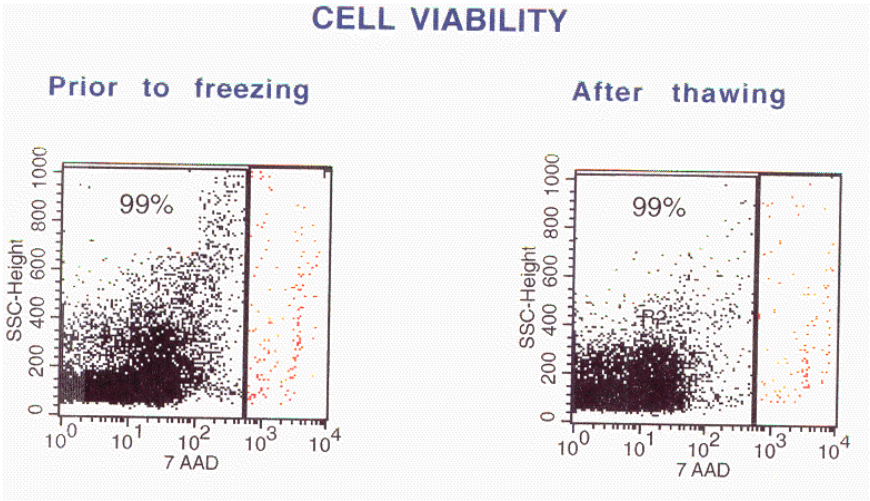
Cell Count:

	NB1	NB2	NB3
BEFORE FREEZING	5.95 x 10^6	4.25 x 10^6	3.35 x 10^6
AFTER FREEZING	2 x 10^6	3.2 x 10^6	3 x 10^6

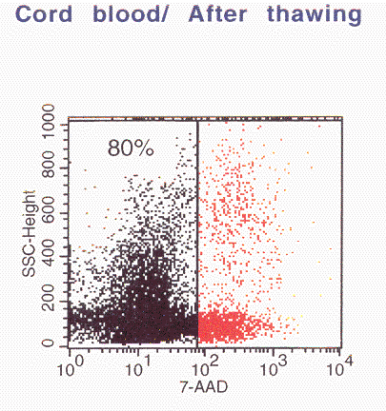
Cell Viability:

	NB1	NB2	NB3
BEFORE FREEZING	97	97	98
AFTER FREEZING	89	98	97

Lymphocytes (figure 1**):**



Stem cells (figure 2)**:**



Cell surface receptors:

Before Freezing

	NB1	NB2	NB3
T Lymphs (CD3+)	75	81	70
T Suppressor Lymphs (CD3+CD8+)	12	24	32
T Helper Lymphs (CD3+CD4+)	61	56	32
NK Lymphs (CD16+56+)	20	9	23

After Freezing

	NB1	NB2	NB3
T Lymphs (CD3+)	72	75	66
T Suppressor Lymphs (CD3+CD8+)	13	21	31
T Helper Lymphs (CD3+CD4+)	60	52	31
NK Lymphs (CD16+56+)	21	11	20

Our results showed that after freezing we could recover about half of the cells. The viability of the recovered lymphocytes was similar to the one of the fresh cells. Also expression of cell surface receptors was not significantly changed after freezing (in most cases <5%).

We have also checked the viability of a cord blood sample after thawing. Cord blood represents an important source of stem cells. As shown in Figure 2, cell viability was 80%.

Discussion:

By looking at the results, we could see that the surface receptors of defrosted and fresh cells were almost the same. This showed that freezing could preserve the surface receptors of the cells. Cell counting showed that not all cells will recover from freezing, but the viability of the recovered ones is similar to the viability of fresh cells.

Freezing was done in the presence of a sulphur-derived chemical (dimethyl sulphoxide) which is believed to maintain good viability of human cells. The reason why a sulphur compound was used comes from previous observations on sulphur derivatives that can block cells from using oxygen and regulate cellular energy production. In fact, earth's earliest life forms began evolving about four billion years ago in an atmosphere without oxygen, but very likely containing hydrogen sulphide. Hydrogen sulphide, a chemical produced naturally by our body, was used to trigger suspended animation in mice by altering the mice from warm-blooded to cold blooded, similar to animals that are hibernating.

Many experiments have been performed to study other species that might or might not enter suspended animation naturally. A recent study showed that zebra fish embryos could be put in suspended animation by cutting down their oxygen level. It is believed that humans could not enter suspended animation by cutting down the oxygen level. The only way humans could be put into suspended animation for now is by dropping the body temperature. Suspended animation will be useful in the medical field.

For example, cancer is a disease that divides the cells rapidly with no limitations. If we find a way to put the malignant cells in the cancer cells to sleep, then cancer might be cured.

Stem cells might be significant when putting human into suspended animation. Stem cells are unspecialized cells that can repair the damaged cells. If we find a way to make a stem cell line that continuously repairing the damaged cells, then we will be able to enter suspended animation for a long period. Stem cells can be used for cloning, since they can evolve into any kind of cells or tissues of our body.

Conclusion:

From this experiment, we found that the human lymphocytes can be preserved under low temperature. Upon defrosting, such cells show high viability and unchanged expression of cell surface proteins. These findings indicate that human cells can be preserved in a frozen state and can be used whenever needed. Since expression of cell surface receptors is not affected, it is likely that the function of these cells will be maintained after defrosting.

We have tested the viability of a cord blood sample after thawing because cord blood represents a very important source of stem cells. Cord blood is obtained at the time of birth from the cord that had connected the baby's body with the mother's placenta. Stem cells are already used for therapy in patients with leukemia, and may have many other applications in the future, such as the treatment of other cancers, diabetes, spinal injuries and many more. We found that the viability of the cord blood sample was quite good, namely 80%.

Whether the human body is capable of entering a state of suspended animation is still a big unknown. However, preserving successfully human cells, as the first step towards this goal, is possible. Having access to frozen cells, such as stem cells, has already great value for human health.

Source:

1) **http://www.daviddarling.info/encyclopedia/S/suspendedanim.html**
2) **National Geographic: Stem Cells, July 2005, p. 2.**
3) **http://news.bbc.co.uk/1/hi/sci/tech/1388622.stm**
4) **Scientific American: Buying Time in Suspended Animation, June 2005, p.50-55.**

ACKNOWLEGEMENT

1) Dr. Adriana I. Colovai.

2) Sue

3) Chuan

4) Dr. Sat

5) Harlem Children Society