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Transporting Mammalian Cells at Ambient Temperature: A Viable Alternative to Dry Ice

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Abstract

The most common method of shipping cells between institutes and companies is sending them frozen, usually treated with anti-freeze solution (most commonly DMSO because it is less toxic than many alternatives), and then packaging them in dry ice for shipment. However many countries place restrictions on dry ice shipments. An alternative to shipping frozen cell vials is to send flasks of growing cells in media. This also has problems because cells in media have limited viability and the flasks can leak. Here we report on an alternative method for shipping viable cells at ambient temperature without dry ice or in media filled flasks. In this study we report on the development and properties of HemSol™. This is an inexpensive, eco-friendly and protects cell integrity at ambient temperature while maintaining viability. We have previously shown that HemSol™ protects platelet and RBC function in cold storage and circulating tumor cells up to 6 days. Therefore we wanted to know if HemSol™ could also be used to transport live cells. Since HemSol™ is a liquid, we experimented with encasing the cells with HemSol™ and gelatin so as to prevent dry ice shipment of cells and circumvent the shipping of cells in media. We performed mock shipping experiments where cells were stored in HemSol™ gel kept at room temperature on a lab benchtop and cells stored in dry ice was also kept on lab benchtop for up to 2 days. After the mock shipping period, we analyzed cells for their functions. Our results show that cells in HemSol™ gel have greater than 95% viability and restored biological functions in 2 hours, whereas, cells shipped in dry ice required more than 24 hours to recover and needed media change to remove the DMSO.

Keywords

HemSol™, HemSol™ Gel, Transportation, Short Term Storage, Cells, Shipment, Dry Ice, Ambient Temperature Shipment

1. Introduction

The standard method of preserving viable cells for long-term storage or transportation requires them to be in suspension, and frozen slowly in the presence of 10% - 20% DMSO and then kept frozen in liquid nitrogen [1]. While freezing is an effective method for long-term on site storage of many types of mammalian cells, shipping them frozen to external locations can be problematic. Shipping cells in liquid nitrogen is hazardous and can be prohibitively expensive. The more economical and less dangerous alternative is to ship frozen cells using dry ice. At atmospheric pressure, liquid nitrogen boils at −196˚C, while sublimation of dry ice occurs at −78.5˚C [2]. Cells can be kept at −78.5˚C for a period, but viability generally decreases and loss of dry ice and thawing of the sample in transit will kill the cells because of DMSO toxicity. Another factor that could impact the frozen shipment of biological samples is the changing regulatory environment in which a number of airlines, couriers and countries are putting increasing restrictions on the use of dry ice for transportation and shipping [3].

In an effort to create an alternative method to dry ice shipping, we formulated a HemSol™ gelatin reagent that allows for shipping of both suspension cell and adherent cell cultures at ambient temperature in gel form, which is not subject to airline regulations. HemSol™ was developed as an alternative method to shipping mammalian cells on dry ice. This reagent is based on a mixture of sugars, which was developed as a storage solution for circulating tumor cells [4] red cells [5] and platelets [6]. HemSol™ contains a patented mixture of FDA approved trehalose, dextran, glucose and mannitol that allows for preservation and shipping of both suspension cell and adherent cell cultures at ambient temperature with improved viability.

2. Material and Methods

2.1. Preparation of HemSol™ Gel

HemSol™ was prepared described previously [4]. Briefly, a HEPES buffer pH 7.4 contained a proprietary ratio of low molecular weight sugars (Trehalose, Mannitol and Glucose) and high molecular weight sugar Dextran was mixed with 10% gelatin (Sigma, St Louis, MO) and maintained at 40˚C to maintain the gel in the liquid form (this mixture is used within 30 minutes). Exposure of the HemSol™ gel to room temperature will cause the gel to form within 15 minutes.

2.2. Cell Lines and Primary Cells

Chinese hamster ovary cells (CHO), human umbilical vein endothelial cells

(HUVEC), colorectal adenocarcinoma cells (CACO-2), Human embryonic kidney cells (HEK293), Heptatocytes, Mesenchymal Stem Cells and B-cells were purchased from ATCC.

2.3. Incubation of Cells and Cell Lines with HemSol™ Gelatin

HemSol™ gel preservation experiments with primary human HUVEC, hepatocytes, B-Cells, mesenchymal stem cells as well as CHO, CACO-2 and HEK293 were performed in collaborations with AscentGene Inc. (Gaithersburg, MD). HemSol™ was mixed with 10% gelatin (Sigma, St Louis, MO) and maintained at 40˚C. Approximately 10^5 - 10^7 cells were kept in their respective growth media prior to treatment with HemSol gel. Live cells were enumerated by trypan blue exclusion. Cells were then mixed with HemSol™ gel and then stored in the solution for the indicated time specified in the experiments described in the Result and Discussion section (typically from 3 - 7 days) at ambient temperature, after which the HemSol™ gel was washed away using the respective cell media and live cells determined using trypan blue exclusion.

2.4. Preparation of Mock Shipping

To prepare for "mock shipping", the suspension cells were grown in complete media. After they reached 80% confluence, they were mixed with HemSol™ gelatin-containing media at 10^5 - 10^6 cells/mL, in which 9 parts of complete media were mixed with 1 part of HemSol™ gel. Adherent cells were grown to 80% confluence in 6 well-plates. Then media was removed and a mixture of 9 parts complete media with 1 part of HemSol™ gel overlaid on top of the cells. Then the cells were left on the bench top for up to 7 days to mimic shipment of samples at ambient temperature.

For dry ice mock shipping. Cells were suspended at 10^5 - 10^6 cells/ mL in a CryoTube™ already frozen by standard methods (ThermoFisher, Waltham MA). Then the vials were removed from the nitrogen tank and placed in a Styrofoam box with dry ice and left on lab bench top for 2 days.

2.5. Functional Permeability Assays with HemSol™ CACO-2Assay with Lucifer Yellow

Caco-2 cells were suspended in commercially available Dulbecco's modified Eagle's medium supplemented with fetal bovine serum, nonessential amino acids, penicillin, and streptomycin (Sigma, St Louis, MO) and then seeded onto 24 transwell membrane inserts at specified cells concentration under a temperature of 37˚C in an atmosphere of 5% CO_2 and 90% relative humidity. The cell monolayers are employed for transport studies 15 - 21 days after seeding between different passages [7].

For permeability assay, transwell contained CACO-2 cells layer were washed with HBSS and 500 µl 0.1 mg/ml Lucifer Yellow in HBSS was added to the apical compartment and 1000 µl HBSS buffer added to basal wells. Plates were incubated in 37˚C incubator for 60 min after which 150 µl samples from basal wells

were removed and read in a fluorimeter with the excitation 485 nm and emission 535 nm. Permeability is given as % of Lucifer Yellow. Less than 3% permeability is acceptable for these studies [7] [8] [9].

3. Results and Discussion

The basic mechanism of HemSol™ gel is to form a solid gel which immobilizes the cells. HemSol™ gel contains cell permeable and impermeable sugars and gelatin that stabilize the cell structure and proteins. When stored at ambient temperature or 4˚C it provides a stable platform for shipping without concerns for spillage or excessive mechanical agitation to the cells. Table 1 shows the results from a number of different cell lines that went through a "mock" shipping process where they were mixed with HemSol™ gel and left at room temperature for up to 7 days. We tested CHO, HEK 293 and CACO-2 cells, for their viabilities and functions after time spent in "mock" shipping.

After 7 days, HemSol™ gel was removed by incubating the plates at 37˚C for 30 min, which liquefies the gel and allows for easy removal and addition of media. Viability was assessed by trypan blue after overnight recovery in a tissue culture incubator. The adherent cells remained attached during this procedure. Suspension cells attached after 2 hours and restored biological functions whereas, cells shipped in dry ice required 1 - 2 days to recover but yielded similar viability (data not shown).

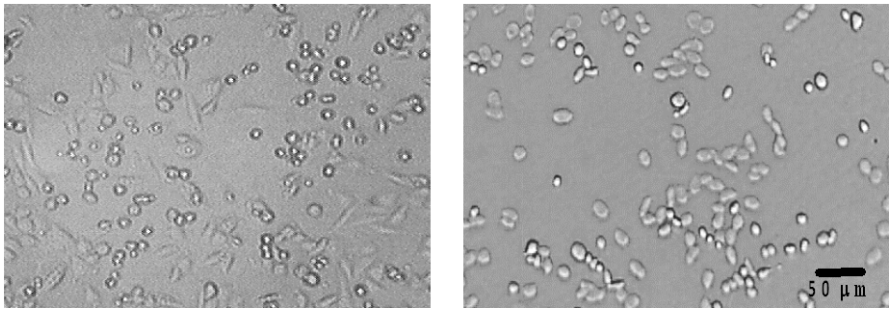
We also examined the ability of CHO cells in suspension to adhere to the plate after 7 days in HemSol™ gel versus CHO cells kept in dry ice for 2 days. CHO cells from both treatments were washed and then plated onto a plate. Figure 1 showed that after 2 hours incubated at 37˚C, CHO cells stored in HemSol gel started to adhere to the plate. In contrast, for CHO cells stored in dry ice, after 2 hours in 37˚C incubator, the cells have not attached to the plate.

We also examined the preservation property of HemSol™ gel for primary cells. In general, primary cells are not as robust as established cell lines that have been grown in tissue culture for years and even decades. However, we performed several pilot studies examining whether HemSol™ gel could be used short-term preservation of primary human cells. Table 2 shows the results of storing various primary human primary cells kept in HemSol™ gel at ambient temperature.

We also determined whether or not, HemSol™ gel can be used to preserve CACO-2 for transportation. CACO-2 cells, which are a human epithelial colorectal adenocarcinoma cell line. When grown on Transwell inserts, they form a

Table 1. Cell lines that were used in mock shipping using HemSol™ gel. Viability was determined by trypan blue exclusion. Each condition was performed in triplicate.

Cell Type	Culture Condition	% Viability after 7 Days in Mock Shipping
CHO	In Suspension	89.9
CHO	Adherent	92.2
HEK293	In Suspension	81.2
CACO-2	Adherent	92.2



(a) (b)

Figure 1. Attachment of CHO cells after 2 hours in 37˚C incubator (a) Cells treated in HemSol™ gel for 7 days at RT versus (b) Cells stored in dry ice for 2 days. Cells were examined under a microscope using 40X magnification.

Table 2. Viability of human primary cells in HemSol™ gel at ambient temperature. After the indicated days in HemSol™ gel, cell in suspension or cells adhered to the plates were warmed to 37˚C to liquefy the HemSol™ gel. Cells were then washed twice with complete media and kept overnight (O/N). Viability was determined by trypan blue exclusion. Each condition was performed in triplicate.

Human Cell Type	Culture Condition	Total Time Spent in HemSol™ gel (Days)	% Viability after O/N Incubation at 37˚C
HUVEC	In Suspension	3	98.2
HUVEC	Adherent	3	96.8
Heptatocytes	Adherent	2	98.9
Mesenchymal Stem Cells	In Suspension	3	96.7
B-Cells	In Suspension	4	98.9

polarized epithelial cell monolayer that provides a barrier to the passage of ions and small molecules. Because of these properties, they are widely used to study intestinal drug absorption.

Using the standard Lucifer Yellow permeability assay [8] [9], we showed permeability of confluent and Lucifer Yellow impermeable CACO-2 monolayers on Transwell inserts treated with HemSol™ gel. The cells were kept at ambient temperature in HemSol™ gel for up to 7 days, after which HemSol™ gel was liquefied, removed and replaced by standard media and then incubated at 37˚C overnight (O/N) in a tissue culture incubator. As shown in **Figure 2**, the permeability of CACO-2 monolayers was still within 1% even after 7 days of storage in HemSol™ gel.

4. Conclusions

Altogether, unlike dry ice, ambient temperature shipping is a novel way to transfer and ship cells. For example, instead of sending cells packaged in dried ice using a styrofoam box, cells can be shipped in a standard box at room temperature. Another key aspect is the environmental and regulation aspect that must be taken into consideration. Styrofoam is non-eco friendly and shipping in dry ice is disallowed in many locations.

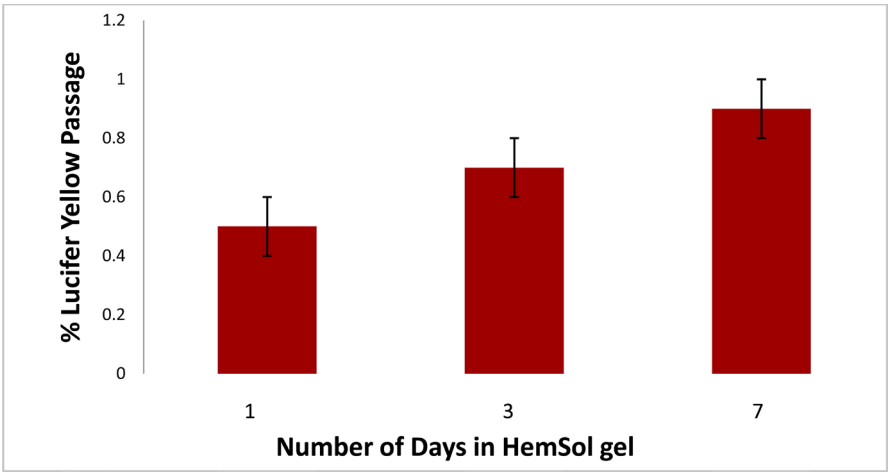


Figure 2. Permeability of CACO-2 monolayers tested by Lucifer Yellow permeability at days 1 through 7. The permeability of CACO-2 kept in HemSol™ gel to Lucifer Yellow was tested after removal of HemSol™ gel, changed the media and incubating at 37˚C overnight.

From a development perspective, a truly "ready to go" cells system for high through put application is now possible. Cells can be prepared in a multi-well plated, packaged with HemSol™ gel and send to end users. Within a day or so, end users will have functional cells already plate to perform assays. Thus, HemSol™ gel is an attractive alternative to standard dry ice shipment and could expand the utilization of cells in pharmaceutical and biotechnology applications.

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