IJHOSCR

Original Article

International Journal of Hematology- Oncology and Stem Cell Research

Defining Permissible Time Lapse between Umbilical Cord Tissue Collection and Commencement of Cell Isolation

Khushnuma Cooper, M.Sc; Viru Shah, B.Sc; Nupur Sapre, B.Sc; Ekta Sharma, M.Sc; Chetna Mistry, B.Sc; Chandra Viswanathan, M.D, Ph.D.

Regenerative Medicine Group, Reliance Life Sciences Pvt. Ltd., Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane-Belapur Rd. Rabale, Navi Mumbai – 400701, Maharashtra, India

Corresponding author: Dr. Chandra Viswanathan
Reliance Life Sciences Pvt. Ltd., Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Thane-Belapur Rd., Rabale, Navi Mumbai – 400701, Maharashtra, India
Email: chandra.viswanathan@ril.com
Tel: 91-22-67678352
Fax: 91-22-67678099

Received: 10, Jul, 2013
Accepted: 1, Aug, 2013

ABSTRACT

Background: Umbilical cord tissue is a very rich source of mesenchymal stem cells. Instead of discarding this source we are banking the tissue along with cord blood for possible future cell based applications. The cord tissue needs to be transported and stored properly in order for it to be good enough for cell isolation at a later date. In this paper we have carried out a validation study to determine the maximum permissible time between cord tissue collection and beginning of cell culture process under defined conditions of temperature and collection media.

Methods: Ten cord tissue samples were used for this study. The umbilical cord tissue segments were transported and stored at 2 – 8^{oC} for varying periods of time viz. 04, 11, 22 and 30 days in a defined medium after which MSCs were isolated and characterized by flow cytometry. Karyotypic studies were also performed on the isolated cells at the above time points.

Results: MSCs could be successfully isolated from 09 even samples after a storage period of 22 days and from 07 samples after a period of 30 days from the date of collection. There was no change in the morphology, immunophenotye, karyotypye and growth potential of the cells isolated from cord tissue after the maximum storage period of 30 days.

Conclusion: The umbilical cord tissue is stable for as long as 22 days if stored at the recommended storage conditions of 2 – 8^{oC} in the defined medium.

KEY WORDS: Adult stem cells, Umbilical cord tissue, Mesenchymal stem cells, Transportation

INTRODUCTION

Over the past few years there has been considerable increase in research on adult stem cell therapies. Mesenchymal stem cells (MSCs) are an attractive tool for stem cell researchers due to their ability to self-renew and differentiate into many different cell types such as osteoblasts, chondrocytes and adipocytes in vitro.[1-3] They are being tried for the treatment of various neurological disorders, cardiovascular diseases, autoimmune diseases and many more. The immense potentials of these cells for future regenerative medicine applications can therefore not be overlooked. MSCs can be isolated from various sources like bone marrow, umbilical cord blood, umbilical cord tissue and adipose tissue.[3,4] Umbilical cord tissue as a source of mesenchymal stem cells was first reported by McElreavey et al.[5] Umbilical cord tissue

provides a unique source of MSCs with immense potential for tissue repair.[6-8] A major advantage of the umbilical cord tissue is its ready availability, no ethical issues and ease of collection thus avoiding invasive procedures. The plasticity of umbilical cord mesenchymal stem cells (UCMSCs) has been previously demonstrated by our group.[9] UCMSCs can be expanded, are remarkably stable, and do not trigger any immune response. Immune modulatory capabilities after allogenic transplantation indicate yet another enormous comfort for the clinical use of MSCs in regenerative medicine applications. The plasticity and expandability of these cells even after prolonged cryopreservation, forms the basis of long term banking for future use. This has led to the establishment of several banks storing UCMSCs with or without cord blood for possible potential use in the future.

For banking, the umbilical cord tissues need to be transported from the collection facilities to the storage site for processing. Appropriate methods for collection, transportation and storage of the cord tissue are required to retain stem cell recovery and maintain tissue integrity and viability. Such tissue banking facilities receive samples from many collection centers. Samples are usually transported within the cities by courier agencies and then delivered to the processing facility. Due to regulations on transportation of biological material there could be delays at the airport which in turn will lead to further delay in receipt and processing of the sample. Other challenges are poor connectivity, infrastructure and communication issues. In those cases where the cord blood collected along with the cord tissue is not suitable for banking due to various reasons such as low blood volume or low cell count, the cord tissue if collected, transported and stored well could address some of the cell therapy needs.

In this paper we have tried to validate the maximum permissible time for which the cord tissue can be stored at 2 – 8^{oC} before commencement of stem cell isolation. The cord tissues were collected in a defined collection medium and transported under validated cold chain conditions. We have used a simple and easily available cord tissue collection media which will maintain the cord tissue viability in case of delays in transportation.

MATERIALS AND METHODS

Cord Tissue Collection and Transport

Umbilical cord tissues were collected from full term births after obtaining an informed consent from the donor mother. Approximately 12cms of the cord was cut and transferred into a labeled tube containing 20ml of collection media (Fig 1). Cord tissue collection medium consisted of phosphate buffered saline (PBS) supplemented with 10% antibiotic solution viz. penicillin, streptomycin and amphotericin B (Lonza Cat.No.17-745E). The tubes containing the cord tissue were placed in a validated collection kit and transported to the processing facility at 2 – 8^{oC}. At the processing facility, the sample was accessioned and a unique barcode was generated that enables tracking throughout the study.

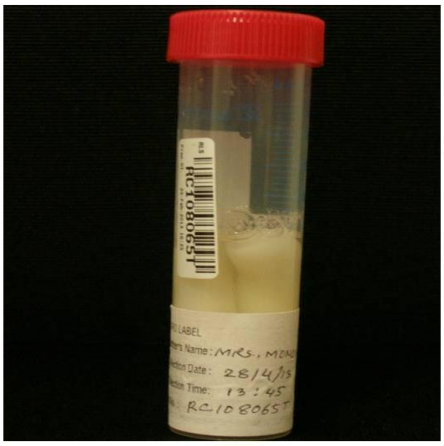

Fig. 1: Cord tissue samples were collected in 50ml tubes containing PBS supplemented with antibiotics. All samples were accessioned and a unique bar code was generated that allowed tracking of the sample.

Infectious Disease Testing

The maternal blood was screened for routine transfusion transmissible disease markers such as HIV I & II and HCV antibodies, HbsAg, Syphillis and CMV IgG and IgM as outlined in the Indian regulatory guidelines.

Processing of Cord Tissue

A total of ten cord tissues were used for the study. After reaching the facility the collection tubes containing the cord tissue segments were removed from the collection kit and stored in a calibrated

refrigerator that maintained a temperature of 2–8^{oC}. The cord tissue was stored in the refrigerator for varying time points viz. 04, 11, 22 and 30 days. At every time point 2 – 3 cms of the cord tissue was cut and taken for processing, the remaining tissue segment was placed back into the same collection tube. The cord piece (2 – 3 cms) was washed with PBS supplemented with antibiotics viz. penicillin, streptomycin and amphotericin B. Twelve to fifteen tissue explants of 1-2mm size were plated in 150mm tissue culture grade Petri dishes (Nunc, Cat. No. 168381) containing DMEM/F12 (Gibco, Cat. No. 11330-032) with fetal bovine serum (Hyclone, Cat. No. SH 30070.03) supplemented with FGF (R&D Systems, Cat. No. 234-FSE) (Fig 2).

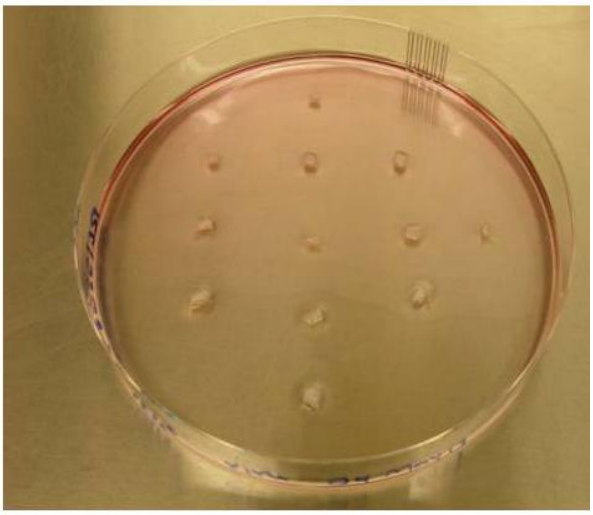

Fig. 2: Twelve explants were plated in tissue culture grade petri dishes for isolation of UCMSCs

The dishes were left undisturbed in a 5% CO2 incubator maintained at 37^{oC} for 4 – 5 days after which fresh culture medium was added to the dishes. Adherent cells were allowed to expand for 10 – 15 days by changing the media at an interval of 48 – 72 hrs. Upon attaining confluence of 80 – 90% the cells were harvested using Tryple Select (Gibco Cat. No. 12563-011). A cell count was performed after harvesting the cells and about a million cells were replated into 225 cm^2 tissue culture flasks (BD Falcon, Cat No.353138) for further expansion up to passage 1.

Immunophenotyping of UCMSCs Isolated at Different Time Points

UCMSCs isolated from cords stored for 04, 11, 22 and 30 days were harvested using Tryple Select and approximately 1x10^6 cells from P0 and P1 were used for immunophenotypic analysis. Cells were characterized for the expression of following markers: CD73, CD105 and CD45 conjugated to either PE or PerCP flurochromes. Non specific fluorescence was determined by staining the cells with directly conjugated isotype matched anti-mouse monoclonal antibodies. All antibodies were purchased from BD Pharmingen except CD105, which was purchased from Caltag Laboratories.
After incubation for 20 minutes at 4^{oC}, the cells were washed with PBS and acquired using a FACS calibur flow cytometer (Beckton Dickinson). Cell viability was also determined by staining the cells with 7-AAD (7 Amino Actinomycin D). Approximately 10,000 events were acquired and data analysis was performed using the CellQuest software.

Pluripotency Assay

UCMSCs isolated from cord tissue samples which were processed after 04 days and at the last time point namely, 30 days of collection were checked for their ability to differentiate into chondrogenic and neuronal lineage.

A-Chondrogenic Potential of UCMSCs Isolated at Different Time Points

To induce chondrogenic differentiation, 0.5x10^6 UCMSCs were seeded in a 15 ml polypropylene tube and were maintained in commercially available chondrogenic medium (Lonza, Cat. No. 3003) supplemented with TGFβ3. The tubes were kept in 5% CO2 incubator at 37^{oC}. Media was changed regularly and the cells grew as pellet cultures. The pellets were collected after 21 days, fixed in 10% buffered formalin and embedded in paraffin. Sections of 4 – 10µm thickness were made for immunohistochemical studies. Sections were stained with Alcian Blue.

B-Neuronal Differentiation of UCMSCs isolated at Different Time Points

For neuronal differentiation the UCMSCs were expanded in cell culture media which consisted of DMEM/F12 supplemented with fetal bovine serum and FGF. Once confluent; this cell culture media was replaced with neural proliferation media as per previously published protocol.[10] UCMSCs were maintained in this neural proliferation medium for one week. After one week the UCMSCs were induced for neural differentiation by addition of butylated hydroxyl anisole (BHA) to the media. The induced cells were checked for the expression of neural markers such as nestin, βTubulin and Tyrosine Hydroxylase (TH) by immunofluorescence studies. All neuronal antibodies were purchased from Chemicon.

Karyotyping Analysis of UCMSCs Isolated at Different Time Points

UCMSCs isolated from cord tissue samples processed after 04 days and 30 days of collection were incubated with growth medium containing 0.25mg of colcemid. After 4 hours of incubation the cells were harvested and resuspended in 0.075M KCl and then fixed in 3:1 methanol/acetic acid.[11] GTG banding was done on metaphase spreads obtained from cultured UCMSCs. Cells were analysed under a Olympus AX70 microscope and karyotyped using Cytovision software.

Statistical Analysis

Cell counts and viability of UCMSC isolated at different time points were compared using Student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Growth Kinetics of UCMSC Isolated at Different Time Points

All maternal blood samples were negative for infectious disease markers. Of the ten cord tissue (CT) samples processed at every time point, except CT 3 all other samples showed growth of UCMSC up to 22 day time point. On day 22 CT 3 did not show cell growth and thus did not grow on day 30 also. After 30 days of storage cord tissue samples CT 3, 4, and 6 did not show cell growth whereas samples CT 1, 2, 5, 7, 8, 9, 10 i.e 7/10 samples showed good cell growth (Table 1). UCMSCs started to migrate out from the cord tissue explants within 15 - 20 days of plating and formed a monolayer of adherent cells which were harvested after a period of 25 days (Fig. 3). We counted the cells at passage 0 for each time point and the average cell counts obtained were $3.2x10^6 \pm 1.2x10^6$, $4.4x10^6 \pm 3.0x10^6$, $4.4x10^6 \pm 2.8x10^6$, and $2.8x10^6 \pm 0.8x10^6$ after 04, 11, 22 and 30 days of storage respectively. Although there were some variations in the cell counts the numbers were comparable to each other at a particular time point. UCMSCs obtained at passage 0 were expanded in 225 cm^2 tissue culture grade flasks and the average cell counts obtained at passage 1 for different time points are shown in Table 2.

Table 1. Cell Growth Potential of Cord Tissues at Various Time Points

Sample No	Day 4	Day 11	Day 22	Day 30
CT 1	+	+	+	+
CT 2	+	+	+	+
CT 3	+	+	-	-
CT 4	+	+	+	-
CT 5	+	+	+	+
CT 6	+	+	+	-
CT 7	+	+	+	+
CT 8	+	+	+	+
CT 9	+	+	+	+
CT 10	+	+	+	+
Summary	10/10	10/10	9/10	7/10

+ = Cell growth observed, **-** = No cell growth observed

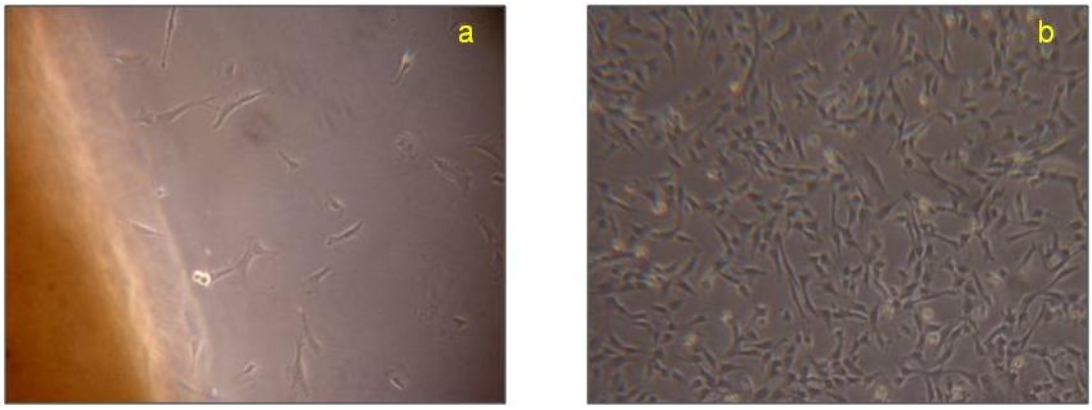

Fig. 3: (a)UCMSCs started to migrate out from the cord tissue explants within 15 – 20 days and formed a monolayer of adherent cells (b).

Table 2. Cell Counts and Immunophenotyping Data of UCMSC Isolated at Different Time Points

Time Point	Passage No	Cell Count (x10^6)	Purity (%)	Viability (%)
Day 4 (n=10)	P1	75.2±43.6	97.3±1.8	87.0±8.0
Day 11 (n=10)	P1	84.9±48.3	97.9±1.3	90.9±7.5
Day 22 (n=9)	P1	46.2±22.3	97.9±2.3	88.6±6.2
Day 30 (n=7)	P1	44.4±18.7	93.4±6.8	93.7±3.5

Immunophenotyping of UCMSCs at Different Time Points

At every time point over 90% of cells were uniformly positive for CD73PE and CD105PE and negative for haematopoietic marker CD45PerCP (Fig 4). Viability of the cells was also found to be more than 80% at every time point (Table2). Although there was a significant decrease in cell counts between cord tissues processed after 04 days and those processed after 30 days of collection (p = 0.0016), the cell viability was not significantly different between the two groups (p = 0.14).

Chondrogenic Potential of UCMSCs Isolated at Different Time Points

UCMSCs isolated after day 4 and day 30 formed pellets within 24 hrs of culture in chondrogenic medium. The cell pellets were collected after 21 days for histological studies. Pellets demonstrated the presence of cartilage specific proteoglycans which was confirmed by Alcian Blue staining (Fig 5).

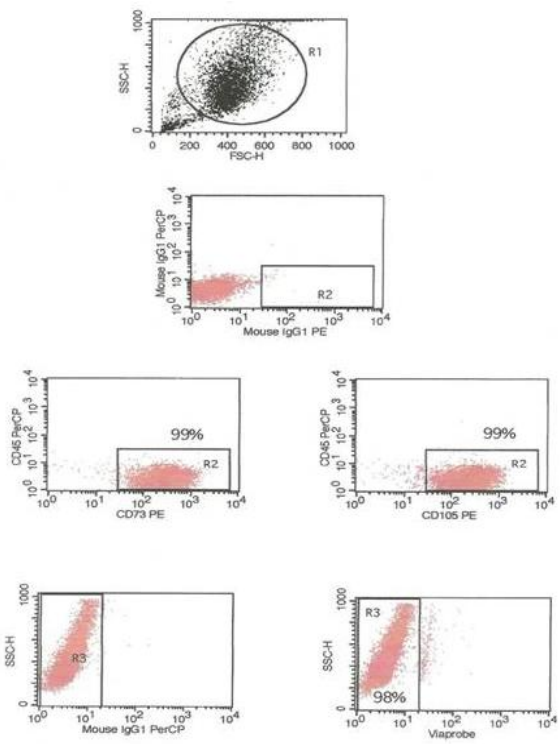

Fig. 4: Immunophenotyping of UCMSCs. The isolated UCMSCs expressed a phenotype of CD73+/CD105+/CD45-

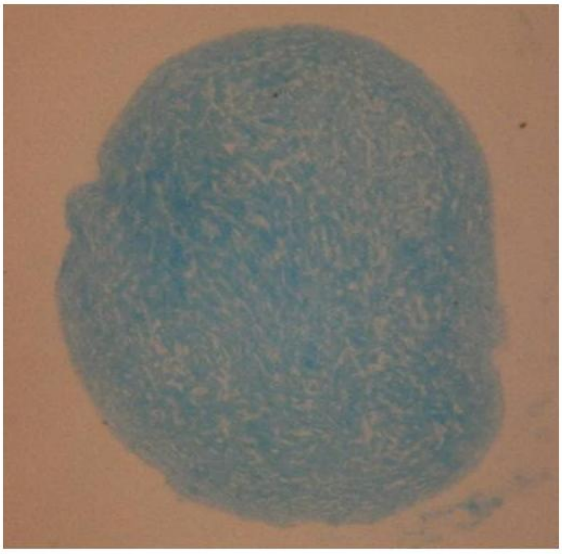

Fig. 5: UCMSCs maintained the chondrogenic potential which was confirmed by Alcian Blue staining

Neural Differentiation of UCMSCs Isolated at Different Time Points

After induction with BHA, the morphology of UMSCs changed within 24 hrs. The differentiated cells showed a neuronal like morphology with extended processes and immunofluorescence studies on these cells also revealed good expression of βTubulin and TH (Fig 6). Expression of nestin was less as compared to the other markers; this was expected as nestin is expressed during early stages of neuronal differentiation and is down regulated upon differentiation.

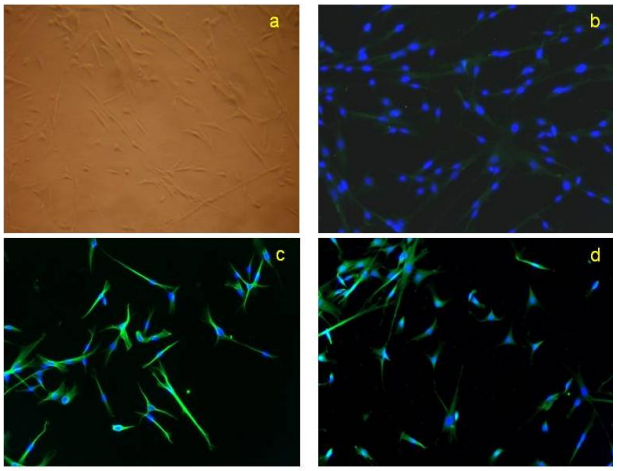

Fig. 6: Neuronal differentiation of UCMSC.
(a) UCMSCs showed neuronal like morphology after induction with BHA
(b – d) Expression of nestin, β tubulin and TH upon differentiation

Karyotyping Analysis of UCMSCs Isolated at Different Time Points

Cytogenetic analysis was performed for UCMSCs isolated from cords processed after the minimum and maximum time points namely, day 04 and day 30 of collection. All samples presented a normal karyotype (Fig 7).

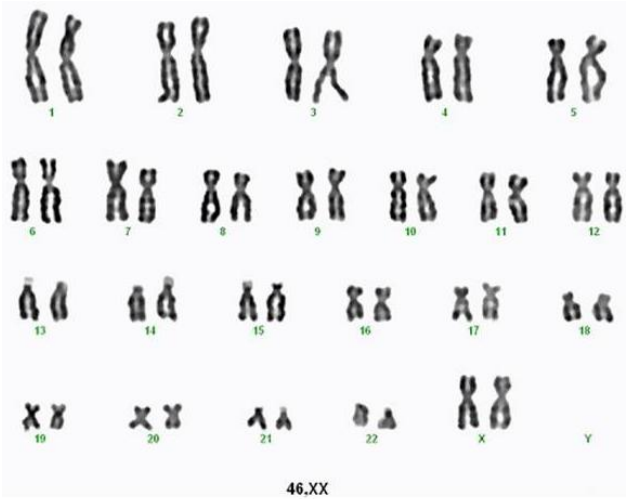

Fig. 7: UCMSCs displayed a normal karyotype

DISCUSSION

The umbilical cord tissue is a valuable source of MSCs. MSCs have been isolated from different compartments of the umbilical cord such as; the epithelium, subendothelium and perivascular area of the umbilical vein, as well as the Wharton's jelly.[12-17] UCMSC banking has been accepted as a way to bank MSCs for possible future regenerative medicine applications. There are several banks offering these services worldwide. Before establishing a UCMSC banking program for cellular therapies one must have validated procedures for transportation of biological samples, cryopreservation and shipping of stem cells to transplant facility. One of the unique problems faced by countries like ours is the ability to transport biological samples in a speedy manner so as to avoid cell loss due to tissue degradation and autolysis. Netcord – Foundation for the accreditation of cellular therapy has published guidelines for transportation and shipping of cellular therapy products. According to the

guidelines all cellular therapy products should be packaged to protect it from potential harm during transit and to prevent exposure of individuals to potentially infectious agents during transport. Primary containers should be placed in a secondary sealed container such as a zip type bag and the ideal transport temperature should range from 2 – 24^{oC}. For our study we have collected the cord tissue in polypropylene tubes and these tubes were placed in a zip lock bag before being transferred into the validated container.

Most of the published studies have shown the isolation of UCMSCs from cord tissue collected in either PBS or Hanks Balanced Salt Solution (HBSS). The maximum time for which the cord tissue has been stored is 48 hrs before commencement of cell isolation. Romanov et al., have collected and processed 26 cord tissue samples within 6 – 12 hours of delivery. They have successfully isolated MSC like cells from the sub endothelial layer of the umbilical cord vein and these cells could be further differentiated into adipogenic and osteogenic lineages.[18] Kadivar et al., have isolated UCMSCs from cords within 6 – 12 hrs of collection. For their study the cord tissue samples were transported in PBS supplemented with a cocktail of antibiotics such as; penicillin, streptomycin, gentamycin and fungizone. They have also shown the cardiogenic differentiation potential of these UCMSCs.[19] Wang et al., have collected the cord tissue sample (n=30) in HBSS and processed the cord tissue samples for isolation of MSCs within 24hrs of collection. The isolated cells were capable of differentiation into the mesodermal lineage.[20] Christodoulou et al., have also collected and transported the cord tissue in HBSS containing 1% penicillin/streptomycin solution, and cell isolation was carried out within a maximum of 48 hrs from tissue collection. Osteogenic differentiation potential of these cells was also shown.[21] Gartner et al., have carried out a study to determine the optimal media for storage and transportation of umbilical cord tissue.[22] They have used four different sterile solutions namely 0.9% NaCl, AOSEPT®-PLUS, PBS and HBSS. The cord tissues (n=12) were stored in these solutions for a period of 7 days at room temperature (22-24^{oC}) and under refrigerated temperature (4-6^{oC}) after which they were processed for light microscopy. Their study has shown that the best transport media for umbilical cord tissue were HBSS or PBS at a temperature of 4 – 6^{oC} since these media maintained the histological structure of the cord tissues even after a storage period of 7 days. This study also showed that cord tissues immersed for 7 days in PBS or HBSS at refrigerated temperature maintained their integrity comparable to a cord tissue collected and processed for histological analysis immediately after birth. Our present study has also shown that integrity of umbilical cord tissue is maintained when transported at 2 – 8^{oC} in PBS and, addition of antibiotics helps in maintaining the sterility of the cord tissues during transport. This study has helped us understand and establish transportation and storage conditions that will prevent tissue losses and thereby loss of cells. In this study we have tried to define the permissible time lapse that will maintain tissue integrity based on our experience with regards to receipt of tissue samples. In countries like that of ours where there could be unexpected delays, the 30 day storage period was with the assumption that this is a reasonable period to receive samples from within and outside the country. The collection media used for the study was selected on account of its ease of availability, cost effectiveness and stability at 2 – 8^{oC}. No replenishment of the collection media was done during the entire storage period of 30 days. The length of the tissue segments collected was fixed at a maximum of 12 cms so that the entire cord tissue segment is submerged completely in the collection medium during transportation to prevent drying of the tissue, which would lead to loss of viable cells. For this study we have used the explants methodology as this protocol provides an easier and less cumbersome method for isolation of MSC without subjecting them to harsh chemicals or enzymes.

UCMSCs isolated from all the cords at varying time periods showed a homogenous fibroblast like morphology throughout the culture period. The isolated cells showed typical MSC phenotype, they were non hematopoietic (i.e. negative for CD45) and expressed CD73 and CD105 markers. These cells also retained their differentiation potential as can be seen by the pluripotency experiments. UCMSCs isolated from cord tissues that were stored

for 30 days also showed good viability of more than 90% and were also found to have no chromosomal abnormalities. Of the 10 tissue samples studied for cell derivation we observed that 90% of the tissues maintained good integrity up to 22 days of storage and 70% up to one month. This variation in cell isolation could be attributed to biological differences that are ill understood.

Thus our study demonstrated that umbilical cord tissue if collected in the above collection media and transported properly under validated cold chain conditions can be stored for a period of up to 22 days without loss of viable cells. This will help in prevention of loss of opportunity to bank UCMSCs resulting out of delayed transportation conditions.

ACKNOWLEDGEMENT

The authors acknowledge Reliance Life Sciences Pvt. Ltd (www.rellife.com), for providing the infrastructure and financial support to work on this project. We would also like to thank Dr. Prathibha Shetty for providing assistance for the neuronal differentiation protocol.

REFERENCES

1. Bruder S, Jaiswal N, and Haynesworth S. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. Journal of Cellular Biochemistry 1997; 64(2): 278–294.
2. Haynesworth S, Goshima J, Goldberg V and Caplan A. Characterization of cells with osteogenic potential from human marrow. Bone 1992;13(1): 81–88.
3. Pittenger M, Mackay M, Beck C. et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411):143–147.
4. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue. Stem Cells 2006; 24: 1294–1301.
5. McElreavey D, Irvine I, Ennis T and McLean H. Isolation, culture and characterization of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. Biochem Soc Trans 1991; 19: 29(S).
6. Shetty P, Cooper K, Viswanathan C. Comparison of proliferative and multileneage differentiation potential of cord matrix, cord blood and bone marrow mesenchymal stem cells. Asian Journal of Transfusion Science. 2001; 1: 14 – 24.
7. Weiss M and Troyer D. Stem cells in the umbilical cord. Stem Cell Reviews 2006; 2: 155-162.
8. Can A and Karahuseyinoglu S. Human umbilical cord stroma with regard to the source of fetus derived stem cells. Stem Cells 2007; 25: 2886-2895.
9. Cooper K, SenMajumdar A and Viswanathan C. Derivation, expansion and characterization of clinical grade mesenchymal stem cells from umbilical cord matrix using cord blood serum. International Journal of Stem Cells. 2010; 3 : 119-128.
10. Shetty P, Thakur A and Viswanathan C. Dopaminergic cells, derived from a high efficiency differentiation protocol from umbilical cord derived mesenchymal stem cells alleviate symptoms in a Parkinson's disease rodent model. Cell Biol Int. 2013;37: 167–180.
11. Kim J, Kim S, Park S, Kim Y, Kim M, Lee M, Ryu H. Mesenchymal progenitor cells in the human umbilical cord. Ann Hematol 2004; 83: 733-738.
12. Covas D, Siufi J, Silva A, Orellana M. Isolation and culture of umbilical vein mesenchymal stem cells. Braz JMed Biol Res 2003; 36:1179–1183.
13. Sarugaser R, Lickorish D, Baksh D, Hosseini M, Davies J. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. Stem Cells 2005;23: 220–229.
14. Weiss M, Troyer D. Stem cells in the umbilical cord. Stem Cell Rev 2006; 2:155–1663
15. Secco M, Zucconi E, Vieira N, Fogac¸a L, Cerqueira A, Carvalho M, Jazedje T, Okamoto O, Muotri A, Zatz M. Multipotent stem cells from umbilical cord: cord is richer than blood! Stem Cells 2008; 26:146–150.
16. Ishige I, Nagamura-Inoue T, Honda MJ, Harnprasopwat R, Kido M, Sugimoto M, Nakauchi H, Tojo A. Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. Int J Hematol 2009;90:261–269
17. Kita K, Gauglitz G, Phan T, Herndon N, Jeschke G. Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. Stem Cells Dev 2010; 9(4):491–502.
18. Romanov Y, Svintsitskaya V, Smirnov V. Searching for Alternative Sources of Postnatal Human Mesenchymal Stem Cells: Candidate MSC-Like Cells from Umbilical Cord. Stem Cells 2003; 21: 105-110.
19. Kadivar M, Khatami S, Mortazavi Y Shokrgozar M, Taghikhani M, Soleimani M. In vitro cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. Biochemical and

Biophysical Research Communications. 2006; 340: 639–647.
20. Wang H, Hung S, Peng S et al. Mesenchymal Stem Cells in the Wharton's Jelly of the Human Umbilical Cord. Stem Cells 2004; 22: 1330-1337.
21. Christodoulou I, Kolisis F, Papaevangeliou D, and Zoumpourlis V. Comparative evaluation of human mesenchymal stem cells of fetal (Wharton's Jelly) and Adult (Adipose Tissue) origin during prolonged in vitro expansion: consideration for cytotherapy. Stem Cell Int. 2013; doi: 10.1155/2013/246134.
22. Gartner A, Pereira T, Gomes R, Luis Lucia A, Franca M, Geuna S, Armada-da-Silva and Mauricio A. Mesenchymal Stem Cells from Extra-Embryonic Tissues for Tissue Engineering – Regeneration of the Peripheral Nerve. In: Prof. Rosario Pignatello editor, Advances in Biomaterials Science and Biomedical Applications. ISBN 978-953-51-1051-4.