A Practical Method for Remote Collection and Preservation of Umbilical Cord Tissue

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Introduction

The increasing demand for private umbilical cord (UC) tissue banking requires finding ways to maintain the integrity of mesenchymal stem cells (MSCs) during transport and culture.

A development study suggested that microbial contamination control was required, but extended exposure time to an antibiotic/antimycotic (AB/AM) solution should be limited to allow reliable MSC outgrowth of the UC tissue.[1]

Purpose

Refine a method for UC birth tissue samples for MSC culture generation indications with the following specifics:

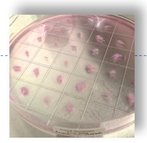
- Methods of procurement at remote sites with shipping to central sites for processing to deliver viable tissue
- Isolation of MSCs with high growth potential and protection from contaminated cultures
- A cryopreservation and storage method that retains viability and flexibility for future uses

Methods

- Umbilical cord segments were collected from consenting mothers according to instructions to the birth provider team by Natera.
- Samples were collected from vaginal (3) and Cesarean (2) births for transport and outgrowth validation studies in 3 conditions.
- Sterile, closed tissue containers were provided for shipment without additives and shipped in a validated shipping box.

Conditions

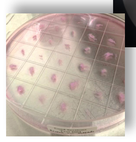
1 Fresh control → Cultured

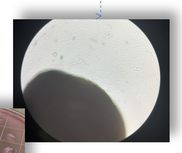


2 **>1 hour** Soaked in AB/AM

Cryo-preserved

3 **>24 hours** → Thawed → Cultured





Growth Evaluated

Culture

Tissue samples dissected - Wharton's Jelly tissue fragments were placed in each square of a 32 square scored, 0.1% gelatin-coated plate.

Tissue samples were cultured using Knockout Dulbecco's Modified Eagle's Medium + 10% FBS (HyClone, Logan, Utah) and PSG (Gibco, Waltham, MA).

Cryopreservation

Tissue was split to allow cryoprotectant diffusion and soaked in CryoStor 10 (BioLife Solutions, Bothell, WA) at 4°C for one hour; was frozen using a step-rate freezer and stored in Vapor phase of LN_2.

Outgrowth

Plates were scored at 21 days as % squares showing growth/squares planted.

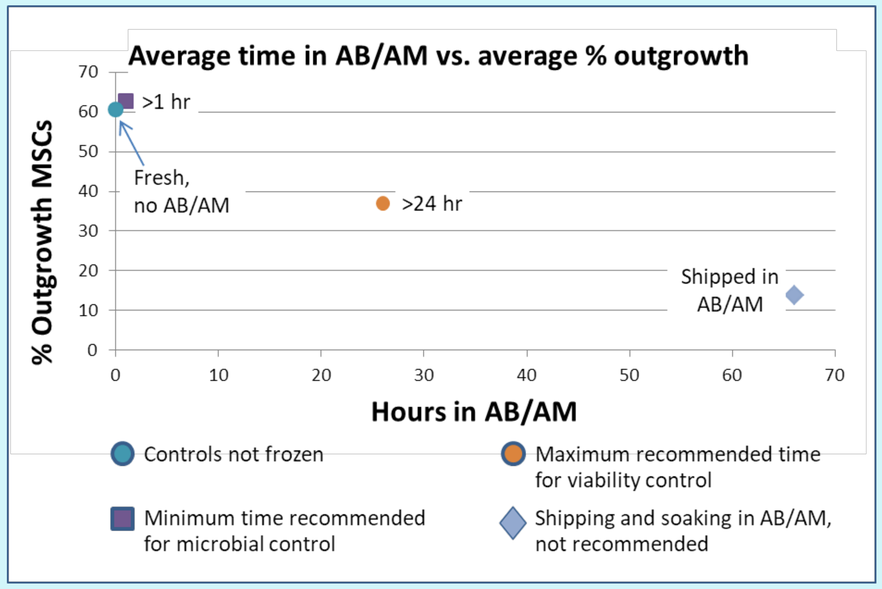
Flow analysis was performed on outgrowth samples sufficient for testing with MSC markers (Miltenyi Biotech, Bergisch Gladbach, Germany).

Flow Cytometry

Outgrowth tissue cells were harvested and analyzed by flow cytometry for CD34, CD73, CD90, and CD105.

Results

- Cord tissue samples were naturally variable with thicker or thinner Wharton's Jelly layers available for culture
- The longer times where tissue was shipped in AB/AM solution were from in the earlier development study[1]
- Cord tissue samples were received 16-31 hours after collection.
- All conditions showed outgrowth
- Comparison of fresh samples and 1 hour AB/AM cryopreserved samples show similar outgrowth.



- The percentage of squares showing outgrowth declined with increasing time of exposure
- All cultured cells analyzed had the following flow cytometry phenotype: CD34 neg, CD73 pos, CD90 pos, CD105 pos

Conclusions

- Shipping UC tissue in a dry, sterile cup with prompt shipment to the processing lab retained the tissue viability/outgrowth potential.[1]
- Cryopreservation medium along with controlled rate freezing and LN_2 vapor phase storage preserved tissue viability without decrement during freeze and thaw.
- Extended exposure to AB/AM solution impaired outgrowth; therefore, laboratory processes should limit exposure time.
- The methods and limits described were effective for delivering UC tissue from the birthing suite to long-term storage with good viability retention.

Discussion

- This method preserved the tissue viability while keeping the integrity of all layers of the collected tissue. In the future, specific tissue elements may need to be isolated for culture.
- MSC outgrowth was the isolated tissue we chose to use as an indicator of viability .
- Human umbilical vein and outer pellicle of the cord remain if future uses for those elements are found.
- Fresh Tissue received in the lab gave the same outgrowth percentage overall as the short AB/AM soaked tissue; longer treatment with AB/AM took a toll on viable outgrowth in our studies.
- Laboratory methods for high volume processing require flexibility to handle tissue from varying locations and situations. These analyses showed dependable viable MSC outgrowth from the cultured tissue that could serve as the start of an autologous tissue culture when exposure times to AB/AM were monitored and shortened.
- Further studies are needed to define precise AB/AM soak time for optimal MSC outgrowth in UC tissue samples compatible with high volume processing to preserve the integrity of all UC tissue layers.

References

1. Haley R, Lehner J, Farrer D. Benson K, and Mathew A. Exploration of shipping conditions to retain viable recovery for umbilical tissue. Abstract presented at 15th International Cord Blood Symposium, June 6, 2017, San Diego, CA.

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