Cost-effective storage solution for delivering umbilical cord with efficient isolation of mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) represent a promising therapeutic candidate for treating many diseases. However, their proliferation and therapeutic abilities decline during the aging process and disease development. Therefore, fetal MSCs derived from the umbilical cord (UC) attract more attention. Storing and delivering the UC is one critical step for efficient MSC isolation. Although the culture medium-based solution is suitable for UC storage, it is not feasible for large-scale preparation because of its high price. Thus, we demonstrate here that a simple solution containing a pH buffering reagent, calcium, magnesium and glucose could be used as a cost-effective storage solution for UC delivery and efficient MSC isolation.

METHOD SUMMARY

The human umbilical cord was cut off within 5–10 min after the baby's birth, stored in a simple solution containing a pH buffering reagent, calcium, magnesium and glucose plus antibiotics on ice, and then delivered to the laboratory. Mesenchymal stem cells were isolated with collagenase B digestion after mincing the umbilical cord into approximately 3 mm³ fragments.

KEYWORDS:

delivering solution • mesenchymal stem cells • storage solution • umbilical cord

Mesenchymal stem cells (MSCs) represent a promising therapeutic candidate for treating many diseases, such as graft versus host diseases [1], cardiovascular diseases [2], diabetes [3] and autoimmune diseases [4–6]. MSCs have both multi-potency and immune modulation capabilities, leading to microenvironment refinement and tissue regeneration [7–12].

MSCs could be isolated from multiple tissues, such as bone marrow, adipose and umbilical cord (UC) [12]. However, their proliferation and therapeutic abilities decline during the aging process and disease development [6]. Thus, fetal MSCs derived from UC (UC-MSCs) [13], composed of embryonic mucous connective tissues [13,14], have attracted more attention during the past decades [13]. Indeed, UC-MSCs have a faster doubling time, higher colony-forming efficiency and greater expansion capacity with longer telomeres than adult MSCs [13,15–19]. Furthermore, as normally discarded after birth, the UC is easily accessible without requiring an invasive procedure and ethical concerns [14,20]. Given these advantages, UC-MSCs are becoming an alternative source of MSCs for preclinical studies and clinical applications to bone marrow-derived MSCs.

However, the standard procedures for collecting, storing and delivering UCs have not been fully developed and demonstrated. Thus, we conducted the present study to develop a cost-effective storage solution for delivering UCs with efficient isolation of MSCs. Our data indicate that a simple solution containing a pH buffering reagent, calcium, magnesium and glucose, referred to as CMGL solution, could be used as a cost-effective storage solution for UC delivery and efficient MSC isolation.

Human umbilical cord collection & delivery

This study was approved by the ethics committee of Shenzhen University (China) and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants. The human UC was cut off within 5–10 min after the baby's birth and stored in the indicated storage solution plus antibiotics (500 units/ml penicillin and 500 µg/ml streptomycin; Thermo Fisher Scientific, MA, USA) on ice and delivered to the laboratory.

Human MSC isolation & expansion

The UC was minced into approximately 3 mm³ fragments and stored in the storage solution for the indicated time. Then, it was incubated in 1 mg/ml collagenase B (STEMCELL Technologies, BC, Canada) for 12 h at 37°C. The fragments were washed with phosphate-

Figure 1. Development of cost-effective storage solution for delivering umbilical cord with efficient isolation of mesenchymal stem cells. The cell number was counted 1 week after the cell plating. The MSCs isolation efficiency was assessed by dividing the counted cell number by the weight of the UC for cell isolation. The experiment was conducted in triplicate. (A) The UC was stored in the indicated solution at 4°C for 6 or 24 h. The MSCs isolated immediately from the UC after the birth (<30 min) without storing in any of the solutions were used as positive controls (Ctrl). (B) The UC was stored in the DMEM or CMGL at 4°C for 6 or 24 h, and then the MSCs were dissociated with collagenase B diluted with DMEM or CMGL. The MSCs isolated immediately from the UC after the birth (<30 min), without storing in any of the solutions and with collagenase B diluted with DMEM or CMGL, were used as positive controls (0 h).

* $p < 0.05$.

Red *: Decreased efficiency compared with controls.

Green *: Increased efficiency compared with controls.

CMGL: Our newly developed cost-effective storage solution containing calcium, magnesium, glucose-low concentration in PBS; DMEM: DMEM/high glucose; HG: High glucose (4.5 g/l); LG: Low glucose (1 g/l); MSC: Mesenchymal stem cell; NS: No significance; UC: Umbilical cord.

buffered saline (PBS), plated onto p12 culture plates in DMEM/high glucose plus 10% fetal bovine serum for 3 days. The fragments were then washed with PBS and replated one more time. The unattached fragments were discarded and the isolated cells were expanded in MSC expansion medium (Dulbecco's Modified Eagle's medium [DMEM]/High Glucose plus 10% fetal bovine serum, 10 ng/ml bFGF) (Peprotech, London, UK), 50 μ g/ml vitamin C (Sigma, MO, USA), 100 units/ml penicillin and 100 μ g/ml streptomycin [5,21].

Human MSC characterization & differentiation

Human MSCs were passaged with TrypLE (ThermoFisher Scientific) for three passages, and then they were subjected to cell surface marker analysis and target differentiation. Differentiation and characterization were performed as described previously [5,21]. Briefly, differentiation was performed with the StemPro[®] Chondro-genesis Differentiation Kit (Gibco, MA, USA), the StemPro Adipogenesis Differentiation Kit (Gibco) and the StemPro Osteogenesis Differentiation Kit (Gibco) according to manufacturer instructions. The cells were differentiated for 28 days and stained with alcian blue for chondrocyte differentiation, oil red O for adipocyte differentiation and alizarin red S for osteocyte differentiation.

Statistical analysis

Data were analyzed using SPSS software for Windows (SPSS Inc., IL, USA) and are shown as mean $\pm$ standard error of the mean. Student *t*-test was used for two-group comparison and one-way analysis of variance for multiple group comparison with normal data distribution, parametric test and Turkey *post hoc* tests. $p < 0.05$ was considered statistically significant.

Although storage solutions for delivering human UC have not been demonstrated in detail, DMEM/high glucose plus antibiotics is the storage solution used most in our laboratory [21], which supports efficient MSC isolation up to 48 h after storing at 4°C or on ice. However, the complicated formulation of DMEM makes it neither cost nor labor effective for large-scale manufacturing. Therefore, it is necessary to develop one simple storage solution for UC delivery with efficient MSC isolation. Theoretically, several basic factors are required, including the pH buffering system, osmotic pressure maintenance, basic nutrients, co-factors for cell attachment and migration. Therefore, PBS was used to maintain the pH and osmotic pressure; glucose was chosen for the basic nutrient; and calcium and magnesium were selected as the co-factors for cell attachment and migration.

To test whether the combination of glucose, calcium and magnesium in PBS could support efficient MSC isolation after storing the UC for a short (6 h) or long period (24 h), their different combinations were compared (Figure 1A & Table 1). Data showed that the solution containing a low concentration of glucose (1 g/l) plus calcium and magnesium in PBS, as the CMGL, was the most efficient solution

Figure 2. Characterization of mesenchymal stem cells isolated from the umbilical cord stored and dissociated with CMGL solution. (A) Cell morphology. **(B)** Cell surface marker analysis by flow cytometry. **(C)** Quantity of CD73⁺, CD90⁺ and CD105⁺ cell analysis by flow cytometry (n = 3). **(D)** Quantity of adipocytes, osteocytes and chondrocytes after differentiation analysis by counting staining positive areas relative to total area among ten different areas (n = 3). **(E)** Representative figures for adipocytes, osteocytes and chondrocytes differentiation of MSCs. CMGL: Our newly developed cost-effective storage solution containing calcium, magnesium, glucose-low concentration in PBS; MSC: Mesenchymal stem cell.

Table 1. Cost and component comparison between CMGL and DMEM (LG).

Components	CMGL (mg/l)	DMEM (LG) (mg/l)
CaCl ₂	200	200
MgCl ₂	95	–
MgSO ₄ ·7H ₂ O	–	200
Glucose	1000	1000
KH ₂ PO ₄	210	–
Na ₂ HPO ₄	385	–
NaH ₂ PO ₄ ·2H ₂ O	–	141
NaCl	9000	6400
Glycine	–	30
L-Alanyl-glutamine	–	862
L-Arginine hydrochloride	–	84
L-Cystine	–	48
L-Histidine hydrochloride·H ₂ O	–	42
L-Isoleucine	–	105
L-Leucine	–	105
L-Lysine hydrochloride	–	146
L-Methionine	–	30
L-Phenylalanine	–	66
L-Serine	–	42
L-Threonine	–	95
L-Tryptophan	–	16
L-Tyrosine	–	72
L-Valine	–	94
Choline chloride	–	4
D-Calcium pantothenate	–	4
Folic acid	–	4
Niacinamide	–	4
Pyridoxine hydrochloride	–	4
Riboflavin	–	0.4
Thiamine hydrochloride	–	4
i-Inositol	–	7.2
Ferric nitrate	–	0.1
Potassium chloride	–	400
Sodium bicarbonate	–	3700
Sodium pyruvate	–	110
Phenol red	–	15
Cost/500 ml (RMB)	16	280

The formulation of DMEM (LG) was obtained from the website of Thermo Scientific company (cat. no. 10567014). The cost was evaluated based on the products made in China, which may vary among different suppliers.

CMGL: Our newly developed cost-effective storage solution containing calcium, magnesium, glucose-low concentration in PBS; LG: Low glucose (1 g/l).

for UC storage and MSC isolation, comparable to the DMEM with low glucose (Figure 1A). Interestingly, the long period of storage in CMGL increased the isolation efficiency of MSCs from the UC (Figure 1A). One potential explanation is that the CMGL might support the proliferation of resident MSCs in the UC. Indeed, the UC matrix contains plenty of growth factors [22], and MSCs are more proliferative at a low concentration (1 g/l) than at a high concentration of glucose (4.5 g/l) [23]. Therefore, CMGL might also be suitable for application in the procedure of cell isolation in addition to UC storage. Normally, to provide efficient nutrients for the isolated MSCs during the isolation procedure, DMEM was used to dilute the dissociation enzyme. The cost would be further reduced if DMEM was replaced by CMGL. Thus, the efficiency of MSC isolation in two dissociation solutions prepared with DMEM or CMGL was compared. Data showed that there was no significant difference between these two dissociation solutions, indicating that CMGL could be used to dilute the dissociation enzyme during the MSC isolation procedure. In summary, CMGL could be used for UC storage and MSC isolation with high efficient MSC isolation.

To further demonstrate that cells isolated with CMGL-based dissociation solution and storage solution for UC delivery have classical characteristics of MSCs, the isolated cells were subjected to morphological cell surface markers and differentiation analysis. Data showed that the MSCs isolated with CMGL-based solutions had fibroblast-like morphology; were positive for CD73, CD90 and CD105; negative for CD45, CD34, CD19, CD11b and HLADR; and capable of differentiating into adipocytes, osteocytes and chondrocytes, with similar levels as MSCs isolated with DMEM/low glucose (Figure 2A–E).

Author contributions

B Lai and J Huang optimized the formulation and performed the MSC isolation. J Chen and W Li collected the samples. H Wu characterized the MSCs. J Xu designed and supervised the project, obtained financial support and wrote the manuscript.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the ethics committee of Shenzhen University and followed the tenets of the Declaration of Helsinki. Informed consent to participate in the study was obtained from participants.

Data availability

All related data are available under request.

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