

ORIGINAL ARTICLE

Medicine Science 2021;10(1):98-100

Effect of short-term in vitro antibiotic administration into the human umbilical cord tissue on cfu count and mesenchymal stem cell immunophenotype **Durmus Burgucu***Akdeniz University Teknokent BabyLife Cord Blood Bank and Human Cell-Tissue Production Center, Antalya, Turkey*Received 04 August 2020; Accepted 18 October 2020
Available online 17.01.2021 with doi: 10.5455/medscience.2020.08.155Copyright©Author(s) - Available online at www.medicinescience.org
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.**Abstract**

Mesenchymal stem cells play important roles in regenerative and reparative medicine applications. Their differentiation and proliferation abilities both in the human body and in the laboratory, environment have been revealed through many clinical and pre-clinical trials. Umbilical cord tissue is an important source of mesenchymal stem cells and has become a popular option in cord blood banking, especially in the last decade. As done for the cord blood, the steps of obtaining, transferring, processing, and cryopreserving umbilical cord tissue are defined by standard procedures. However, there is limited information about the microbiological contamination rate of tissues and their decontamination before the procedure. This study aimed to assess the effect of in vitro short-term antibiotic treatment applied to the umbilical cord tissue obtained via superficial ethanol disinfection immediately after birth on the formation of colony-forming units (CFUs) and immunophenotype of the mesenchymal stem cells. For this purpose, tissues obtained from 10 individuals were evaluated in three different groups that included the control group and groups of tissues with 5- and 10-min antibiotic treatment. There was no significant difference among the groups in the expression levels of CD45, CD34, CD73, CD105, and CD44, which are the markers used in immunophenotyping. Similarly, no difference was found in CFU formation either. The present study has revealed for the first time that an antibiotic preparation used in in vitro cell culture for cord tissue banking can be used safely in tissue preparation and banking stages. Our results show that short-term in vitro antibiotic administration can contribute to the standard formation during decontamination/disinfection stages in cord tissue banking.

Keywords: Umbilical cord, mesenchymal stem cell, cord blood bank**Introduction**

Human umbilical cord blood has been successfully used as a source of hematopoietic stem cells from 1988 to the present day [1]. Transplant of more than 40,000 units of cord blood has been successfully performed in both children and adults for the treatment of several diseases, including hematological, metabolic, immunological, neoplastic, and neurological disorders [2]. Also, the number of units within the cord blood banks has reached about 5 million units worldwide, including 800,000 units in state-owned banks and more than 4 million units in private banks [3]. One of the key points in cord blood banking is the microbiological screening of the obtained blood. Studies around the world have shown that the bacterial contamination rate is 1%–2% [4–5]. Just like umbilical cord blood, umbilical cord tissue has also been a popular

topic in the studies conducted in the field of cord blood banking, especially in the last decade. Cord tissue is an important source of mesenchymal stem cells [6]. As done for the cord blood, the steps of obtaining, transferring, processing, and cryopreserving the cord tissue are defined by standard procedures [7]. However, there is limited information about the microbiological contamination rate of tissues and their decontamination before the procedure. This study aimed to assess the effect of in vitro short-term antibiotic treatment applied to the umbilical cord tissue obtained via superficial ethanol disinfection immediately after birth on the formation of colony-forming units (CFUs) and immunophenotype of the mesenchymal stem cell.

Material and Methods

This study was approved by the Ethics Committee of the Akdeniz University Faculty of Medicine (decision number: 592-date: 11.10.2017), and volunteer participants signed an informed consent form. Removal, transfer, and decontamination of the tissues: The cord tissues of the subjects who applied to the cord blood bank were cleaned with 70% ethanol and then surgically cut and taken into a

*Corresponding Author: Durmus Burgucu, Akdeniz University Teknokent BabyLife Cord Blood Bank and Human Cell-Tissue Production Center, Antalya, Turkey, E-mail: dburgucu@akdeniz.edu.tr

cord tissue transfer kit immediately after birth and delivered to the GMP laboratory. The tissue was first removed from the transfer kit and then was washed in a sterile petri dish (Falcon 100 mm-353003) with normal saline (0.9% ISOTONIC SODIUM CHLORIDE SOLUTION-Polifarma/c-1911002). Thereafter, it was divided into fragments of 0.3 cm. These fragments were treated with normal saline containing a 4% antibiotic solution (P0781 Sigma-Aldrich Penicillin-Streptomycin). A preliminary study was conducted to determine the concentration of antibiotics. We started with 1% in standard cell culture conditions and increased it up to 4%. As short-term implementation was planned, we decided on 4× standard concentration. We used flow cytometry with 7AAD to test that 5 and 10 minutes of the application did not exhibit a negative effect on cell viability (viability >95%, data not shown). In this preliminary study, mononuclear cells obtained from cord blood were used. In summary, a 4% concentration was considered the safe upper limit for this study. Two groups of tissues were kept in the antibiotic solution: one for 5 min and the other for 10 min. For primary culture, the tissue was cultured for 21 days in a complete medium [Dulbecco's Modified Eagle Medium (DMEM Gibco-11965092) +10% autologous plasma]. At the end of the 21st day, tissue fragments were removed. Samples with 70% confluence were divided into two Petri dishes (100 mm). CFU assay: Cells obtained after the primary culture were transferred into 6-well plates so that there well 5000 cells per well and incubated for 2 weeks in the wells containing 3 ml 0.5% noble agar (A5431 Sigma-Aldrich) (37°C, 5% CO₂). Following incubation, the cells were fixed with 10% formalin (HT501128 Sigma-Aldrich) and dyed with Giemsa (GS500 Sigma-Aldrich). The colonies formed were counted. Mesenchymal stem cell immunophenotyping: After the primary culture, cells were collected into a polystyrene tube of 5 mL (BD 352003) so that there were 100,000 cells in each tube. For each sample, three tubes were prepared. Ten microliters of anti-CD34 (BD Pharmingen PE Mouse Anti-Human CD34 Cat No. 560941) and anti-CD-45 (BD Pharmingen FITC Mouse Anti-Human CD45 Catalog No. 555482) were added to the 1st tube; anti-CD44 (BD Pharmingen FITC Mouse Anti-Human CD44 Catalog No 560977), anti-CD-73 (BD Pharmingen PE Mouse Anti-Human CD73 Cat No 550257), and anti-CD105 (BD Pharmingen APC Mouse Anti-Human CD105 Cat No. 562408) were added to the 2nd tube, and isotypic controls (Mouse IgG1, κ) were added to the 3rd tube for each fluorochrome. These were incubated at room temperature in the dark for 20 min. Following incubation, the cells were washed twice with sterile PBS, and flow cytometry (BD Accuri C6) analysis was performed.

Statistical analysis

SPSS 21 statistical software was used. For intergroup comparisons, Kruskal–Wallis H-test was performed. $P < 0.05$ was considered significant.

Results

The cord tissues obtained from 10 individuals were divided into three fragments, and immunophenotypic analysis and CFU assay were performed in three different groups belonging to the same individual (the control group and the groups with 5- and 10-min antibiotic treatment) after the primary culture process. There was no significant difference among the groups in the primary culture stage and later in the CFU assay (Figure 1). This result shows that

short-term antibiotic administration does not harm mesenchymal stem cells in terms of CFU formation. CD45, CD34, CD73, CD44, and CD105 are usually used as markers in the immunophenotyping of mesenchymal stem cells. The expression levels of these markers were assessed as % values in the flow cytometry analysis of mesenchymal stem cells obtained from each group after the primary culture (Figure 2). No significant difference was observed in expression levels when each marker was compared among the groups ($P > 0.05$). This result suggests that antibiotic administration does not affect the immunophenotypic properties of mesenchymal stem cells after the primary culture (Table 1).

Table 1. Immunophenotypic properties of mesenchymal stem cells after the primary culture (n = 10; $P > 0.05$)

	CD73	CD105	CD44	CD34	CD45
control	99	98.33	97.25	0.51	0.48
Antibiotic (5 min.)	99.15	98.36	97.42	0.52	0.51
Antibiotic (10 min.)	99,1	98.08	97.35	0.57	0.51

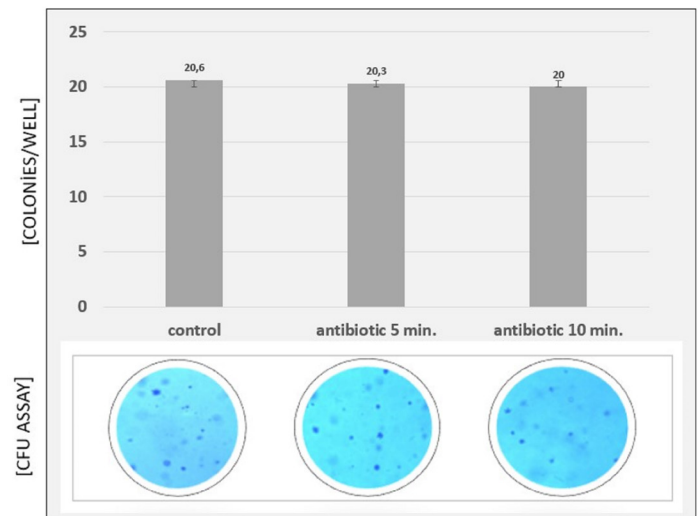


Figure 1. CFU counts: CFU counts of the control group and the groups with 5- and 10-min antibiotic treatment. Values are given as mean values (n = 10; $P > 0.05$)

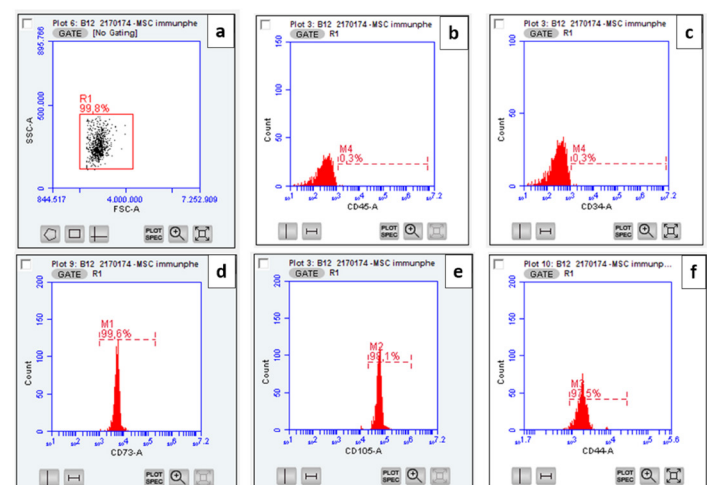


Figure 2. Mesenchymal stem cell immunophenotyping: Flow cytometry histograms

a) SSC/FSC, b) CD45 expression, c) CD34 expression, d) CD73 expression, e) CD105 expression, f) CD44 expression

Discussion

Mesenchymal stem cells play important roles in regenerative and reparative medicine applications. Their differentiation and proliferation abilities both in the human body and in the laboratory, environment have been revealed through many clinical and pre-clinical trials [6-8]. Furthermore, mesenchymal stem cells play important roles in immune system responses via their autocrine and paracrine effects. They exert an immunomodulatory effect, especially in hyperimmune response regulation [9]. Obtaining the cells and storing them under favorable conditions is one of the most important limitations in stem cell and cellular treatments. Successful isolation and reproduction of mesenchymal stem cells in the laboratory is important to produce a cell-based product for therapeutic purposes. Mesenchymal stem cell production is a time-consuming and labor-intensive process. Reproduction time, microbial contamination risks, and possible changes in biological characteristics are processes that need to be managed well. In mesenchymal stem cell production for therapeutic purposes, donor screening and testing, recovery, and safety and efficacy of the cells are important in terms of banking. There are two different studies in the literature regarding cord tissue contamination rate. One of these indicates that the rate is below 1%, especially in cesarean deliveries [10], whereas the other study has reported a contamination rate of 1.22% without indicating the method of delivery [11]. One study has reported in the methodology section that 70% ethanol was used [10], and the other has reported that 75% ethanol was used [11] but no antibiotics were administered. The tissues obtained in the present study were examined in three groups. The tissues obtained from 10 different individuals were grouped into the control group and the groups with 5- and 10-min in vitro antibiotic treatment. To standardize the donors in terms of the method of birth, only those with cesarean deliveries were included in the study. All stages were performed in GMP areas. The reason for this preference was to create a cell bank operating by GMP and to perform the practice according to GMP standards, which is a recommended choice. The approach of using a standard manufacturing process and comprehensive testing guarantees the safety, identity, purity, and strength of the final product [10]. In the present study, it was revealed for the first time that an antibiotic preparation used in in vitro cell culture for cord tissue banking can be safely used in tissue preparation and banking stages. Our results

show that short-term in vitro antibiotic administration can contribute to the standard formation in decontamination/disinfection stages of cord tissue banking.

Financial Disclosure

This study was conducted within the scope of the project numbered 2170174 of the Scientific and Technological Research Council of Turkey (TUBITAK)-1512 Individual Entrepreneurship program.

Ethical approval

This study was approved by the Ethics Committee of the Akdeniz University Faculty of Medicine (decision number: 592-date: 11.10.2017).

References

1. Gluckman E, Broxmeyer HA, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med.* 1989;321:1174-8.
2. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood.* 2013;122:491-8.
3. Mayani H, Wagner JE, Broxmeyer HE. Cord blood research, banking, and transplantation: achievements, challenges, and perspectives. *Bone Marrow Transplant.* 2020;55:48-61.
4. Zhu L, Lv H, Wang Y, et al. Microbial screening of unrelated cord blood units in a Chinese cord blood bank. *Transfus Med.* 2013;23:438-41.
5. Chen SH, Zheng YJ, Yang SH, et al. Microbial contamination of the Tzu-Chi Cord Blood Bank from 2005 to 2006. *Acta Paediatr Taiwan.* 2008;49:9-13.
6. Couto PS, Shatirishvili G, Bersenev A, et al. First decade of clinical trials and published studies with mesenchymal stromal cells from umbilical cord tissue. *Regen Med.* 2019;14:309-19.
7. Dulugiac M, Moldovan L, Zarnescu O. Comparative studies of mesenchymal stem cells derived from different cord tissue compartments - The influence of cryopreservation and growth media. *Placenta.* 2015;36:1192-203.
8. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8:315-7.
9. Gao F, Chiu SM, Motan DA, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis.* 2016;7(1): e2062. Published 2016 Jan 21.
10. Gong W, Han Z, Zhao H, et al. Banking human umbilical cord-derived mesenchymal stromal cells for clinical use. *Cell Transplant.* 2012;21:207-16.
11. Ganesh Kumar Sarvesa, Chirayu Padhiar, Ashika Asha. Comparison study of 70% ethanol effect on direct and retrieval culture of contaminated umbilical cord tissue for expansion of msc at lifecell. *Cytotherapy volume 17, issue 6, supplement, s36, June 01, 2015*