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Page: 60-64

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Case Study

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A simple and quick isolation method to obtain a huge number of fibroblasts like mesenchymal stem cells from human umbilical cord

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Abstract: In this publication, we have developed a simple and quick isolation method of stem cells from umbilical cord. Usually, umbilical cord is a waste product during the child delivery procedure, but the umbilical cords contain valuable stem cells called mesenchymal stroma stem cells (also called MSCs), which can be basis for different applications e.g., testing effect of therapeutic compounds as well as development of therapeutic applications for cure of degenerative diseases like bone and cartilage related. These stem cells can be used to create new artificial organs too. The umbilical cord was cut with sterile scissors into small pieces (1-2 mm size) and these pieces were placed on 6 well culture plates. The cell culture media was added to these plates after 5-10 minutes placing these plates in CO_2 incubator. The successful growth of cells was observed after 7 days in wells, where pieces of umbilical cord were implanted. Microscopic observations show how the cells are oozing out from these pieces. These cells were isolated and passaged further. These adhere on the plastic surface, were fibroblast shaped and isolated successfully in different experiments.

INTRODUCTION

The plastic adherent and fibroblast like cells were isolated from bone marrow in late eighties[1,2]. These cells were called mesenchymal stem cells (MSCs). These cells are in position to differentiate in different lineages like osteogenic, chrondrogenic, adipogenic, myogenic and many others in vitro experiments[3]. MSCs express different markers like CD105, CD90, CD73, but do not CD45, CD34 and HLA-DR markers. MSCs are known to have immunomodulatory effects, hence they may be candidate for allogenic clinical therapies e.g., transplantation in cancer patients as well as autoimmune disorders. MSCs are to be subjected for the possibilities to regenerate bones and cartilages along with non-skeletal tissue applications e.g., generation of cardiomyocytes, spinal cord tissue, pancreatic and hepatic tissue etc, which can lead to cure the many chronic diseases of society. Usually, these MSCs can be isolated from different sources like bone marrow, adipose tissue, joint tissue, dental pulp and other sources[4]. Umbilical cord is one of the waste products during the delivery of a child and usually it is thrown away, but it offers also a good source of MSCs, therefore we develop a simple method to isolate the MSCs in laboratory[5,6].

MATERIAL AND METHOD

The umbilical cord was transferred to laboratory under the cooling condition after consent of donors and it was stored for one day at 4 °C. The whole procedure was conducted under sterile conditions and with sterile instruments. The umbilical cord was cut with sterile scissor in 2-3 cm pieces in such way that blood vessels were avoided and placed in a sterile petri disc. (Picture 1) If there were blood

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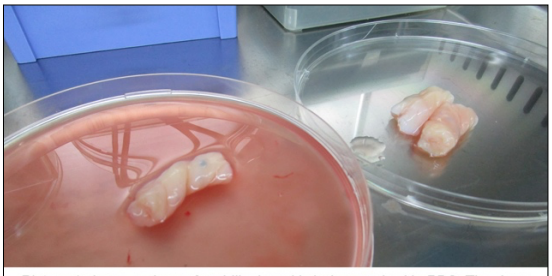
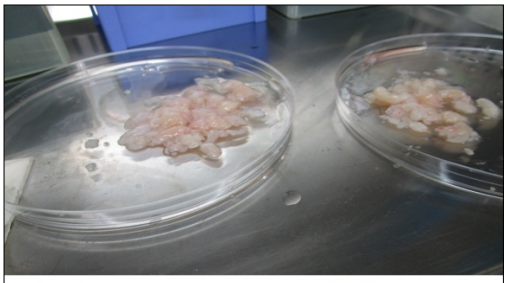
vessels, they were punctured with forceps or scissor to remove the blood. The 2-3 cm piece was washed in PBS (Lonza, USA) twice to remove the blood and other fluids. After that piece of umbilical cord was placed in fresh petri dish in 2-3 ml PBS and cut into small pieces of 2 to 4 mm. (Picture 2) These were collected in 50 ml tubes and treated with 2ml trypsin/EDTA solution (Promocell, Germany) for 30 minutes. A part of pieces was kept untreated. (Picture 3) They were filtered through a retainer to get the pieces at the top of 50 ml tube. The treated and non-treated pieces were placed in wells of 6 well cell culture plate as well as 50 ml cell culture flasks with the help of sterile forceps. These plates without cell culture media were placed in CO_2 inoculator for 5 minutes at 37°C, so that pieces of umbilical cords adhere to the plastic surface of the plate or flask. This step is very important, otherwise pieces of umbilical cord will float in the cell culture media and there will be no stem cells, therefore it is important that umbilical pieces adhere to the plastic surface before the media was added. Each well has only 3-5 pieces only. After this, 5 ml cell culture media DMEM (Lonza, USA) with 1% Streptopenicillin and 1% Glutamine containing 10% fetal calf serum (Biochrome, Germany) were added to each well. This whole above-mentioned procedure took around 60-75 minutes to finish.

The plates and flasks were kept at 37 °C very carefully and gently in CO_2 incubator so that pieces of umbilical cord do not start floating in the media. They were observed under the microscope daily and changed the media on 3rd day. On day 7, there was growth of fibroblast like cells coming out from these pieces and these cells adhere to the plastic surface. On day 14, these cells are removed with trypsin I EDTA treatment and washed in PBS to get the pellet. They were counted under the microscope with Neubauer Chamber with trypan blue dye (Biochrome, Germany). A part of the pellets was passaged in 6 well cell culture plates and 50 ml flasks. The rest pieces of umbilical cord and stem cells were frozen in freezing media (Genekam, Germany) containing DMSO for later use. They were analyzed for expression of specific markers.



Picture 1 shows a piece of umbilical cord is being washed in PBS. The deep red colored spot is a blood vessel in this. Second petri dish shows washed pieces of umbilical cord, which were cut into small pieces to isolate the stem cells.



Picture 2 shows that umbilical cord is cut in small pieces of 1-2 mm in two petri dishes

RESULTS AND DISCUSSION

There was successful growth of fibroblast like cells in cell culture wells and flasks. The pictures are showing that the cells were oozing out from the small pieces of human umbilical cords. (Picture 4 and 5) Trypsin/EDTA treated umbilical cord pieces provided more fibroblast like cells, which adhere to the plastic surface. Such cells were also observed in plates in non-treated umbilical cord pieces. There was need of only 3 to 5 pieces of umbilical cord in each well and in the flasks. (Picture 6) It shows that umbilical cord is huge source of MSCs because 3 cm piece has generated huge number of cells. From 3 cm piece of umbilical cord, we were able to get 2 plates with 6 wells and 3 Flasks. A part of small pieces was stored in freezing media for later use.

Cutting of umbilical cord needed some patience and good scissors as it slips, hence this care must be taken in order to avoid the loss of umbilical cord with this mistake. The small blood vessels in umbilical cord made empty in order to remove the blood cells including erythrocytes because they may be disturbing

factor during the isolation process. Multiple PBS washings were done to remove them.

The counting of stem cells shown that number of cells obtained per well was about one million in treated population, where number of cells obtained from non-treated pieces (trypsin/EDTA) was less. The growth was observed in 12 wells and 3 flasks. No growth was observed, where the pieces were floating in media. (Data not shown)

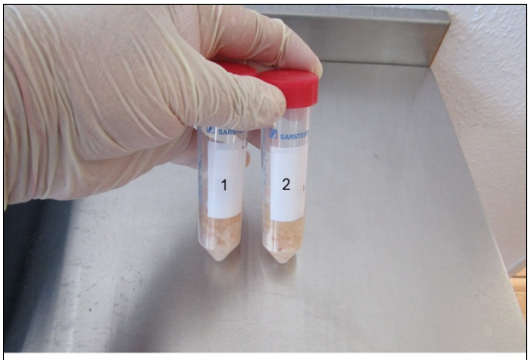
The passaging of the cells was done after the trypsin/EDTA treatment. Once these fibroblast-like cells were treated with trypsin, it was found that they can be easily removed from the plastic surface for further passaging. The cells were able to passage up to 7 passages. After the 7th passage, they lost their activity to redivide and they change their shapes, which become irregular.

They were tested for the presence of biomarkers like CD90, CD73 and CD105 and It was found that they were positive for these. The presence of CD90 marker was used to isolate these cells with magnetic beads along with CD105 marker.

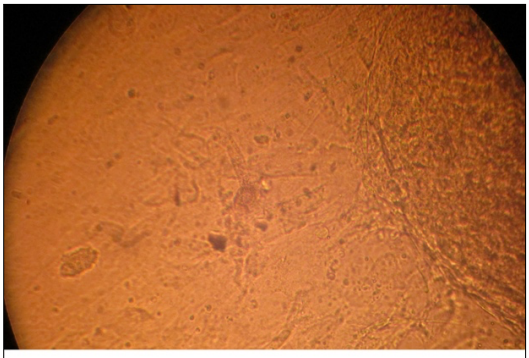
In this research work, we have shown a simple method to isolate the fibroblast stem cells called MSCs in different setting (treated and non-treated). As there is a need of large number of stem cells to conduct the studies, umbilical cord offers a huge source of stem cells. We are able to get one million cells per well as well as per flask, hence there may be many million cells from the whole umbilical cord, which may be solution for a large number of stem cells needed for different therapeutic applications. Average size of human umbilical cord is 55 cm; hence user can isolate many million cells because in this research, 3 cm pieces of umbilical cord were used. These stem cells can be stored for long term for repeated applications. The industry can also isolate these stem cells from umbilical cord and provide to research institutes at discounted prices with constant quality instead each institute is going to isolate them, hence this can result in new research fields. MSCs can be obtained from other sources like adipose tissue and bone marrow, but one cannot obtain such a large number of cells. Moreover, umbilical cord is a waste product and it is discarded after the child delivery. This publication and other research work show that umbilical cord can be a big source of MSCs[3]. Our method opens door to many laboratories around the world to isolate the MSCs in large number to do research in 2D as well as 3D applications using biomaterials for development of new therapies[7]. The stem cells are slowly growing cells, therefore many times, it is very difficult to have the sufficient number as single population to conduct the studies, this shortage of homogenous population may be solved through this method. We have shown in another publication that these cells can be isolated with the help of CD90 magnetic beads and can be passaged from the plates as well as flasks after the trypsin treatment[8].

The supernatant from MSCs is being tested as potential therapeutic option for many diseases e.g., joint disorders because it has immunomodulating properties[9]. The studies are conducted in 2D and 3D applications to find the therapeutic potential on corneal wound[10]. MSCs generated through our method offers an excellent supernatant source in large volume for research community to conduct further studies.

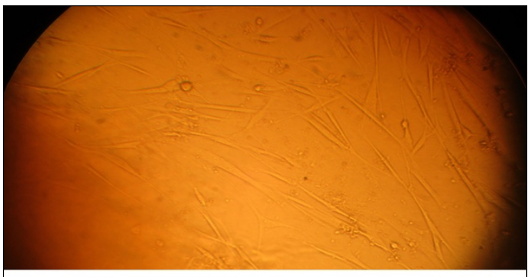
The method presented in this research work is simple and easy to be carried in any laboratory against the method presented in a video publication and other publications[11]. Different methods of isolations in literature show that user needs a lot of time to isolate the stem cells from umbilical cord against our method, which can be finished within 60-75 minutes (around one hour) and a number of plates or flasks can be cultivated with umbilical cord pieces to generate huge number of stem cells[12].



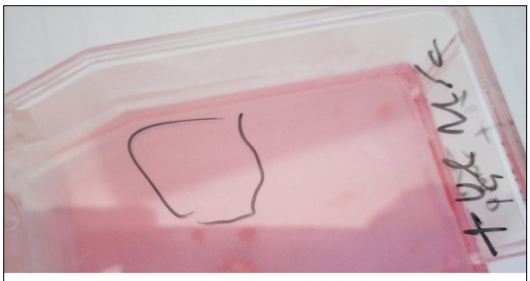
Picture 3 shows that 1-2mm pieces of umbilical cords are treated with trypsin in 50ml tubes. Tube 1 is treated and tube 2 is non treated.



Picture 4 shows under microscope that fibroblast like stem cells are oozing out from pieces of umbilical cord.



Picture 5 shows under microscope that fibroblast like stem cells are oozing out from pieces of umbilical cord.



Picture 6 shows that there are 5-6 pieces (1-2 mm size) of umbilical cord in 50 ml flask, from them the growth of stem cells were observed under microscope.

CONCLUSION

The cells were tested for the presence of biomarkers like CD90, CD73 and CD105 and it was found that they were positive for these. The average size of human umbilical cord is 55 cm and the stem cells can be stored for long term for repeated applications. The industry can also isolate these stem cells and provide them to research institutes at discounted prices with constant quality. MSCs can be obtained from other sources like adipose tissue and bone marrow, but they are a waste product and discarded after the child delivery. The method opens door to many laboratories around the world to isolate the MSCs for research in 2D and 3D applications using biomaterials.

DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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