Isolation of Ovine Multipotent Mesenchymal Stem Cells from Umbilical Cord Tissue Wharton’s Jelly

S. Eswari1, M. Monisha, K. Vijayarani and V.S. Gomathy
Department of Veterinary Physiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600007, Tamil Nadu.

(Received : 19-12-2015; Accepted : 29-02-2015)

Abstract

In this study, we tried explant method and a combination of explant-enzymatic method for the extraction of Mesenchymal Stem Cells from ovine umbilical cord tissue wharton’s jelly. The isolated and culture expanded cells expressed strong alkaline phosphatase activity and they were shown to form colonies. The Mesenchymal Stem Cells showed positive for CD44 and negative for CD34 surface markers by flow cytometry. These findings indicated that the cells with MSC phenotype can be isolated much faster and higher in mixed explant-enzymatic method from ovine UCT-WJ.

Key words: Mesenchymal stem cells, isolation, sheep, Wharton’s jelly

Mesenchymal Stem Cells (MSCs) are attractive candidate for cell-based therapeutic strategies, because of their easy isolation and proliferation potential. The wharton’s jelly (WJ) is the gelatinous connective tissue from the umbilical cord tissue (UCT) and composed of myofibroblast like stromal cells, collagen fibres and proteoglycans (Kobayashi et al., 1998). The stem cells in WJ are expected to become seed cells for tissue engineering, cell therapy and gene therapy (Butler et al., 2000). The MSCs have been obtained from the fetuses (Fuchs et al., 2005), umbilical cord tissue in humans (Venugopala et al., 2011), bovine (Singh et al., 2013; Sreekumar et al., 2014) and in caprine (Moshrefiet al., 2010). Till date, no investigations were found about different methods for isolation of MSCs in order to obtain the cells at much faster rate. Therefore in the present experiment, the isolation of MSCs from ovine UCT-WJ by two different methods were studied.

Materials and Methods

Umbilical cords were separated from the foetuses obtained from the abattoir at Perambur, Chennai and washed in sterile Phosphate buffered saline (PBS) with 200IU/ml penicillin and 200µg/ml streptomycin (Figure 1A). The WJ (Figure 1B) was cut into small pieces of 1 cm³ and cultured as explant in tissue culture flask (Figure 1C). For mixed explant–enzymatic culture method, the WJ pieces were digested in enzymatic cocktail comprising collagenase type I (4mg/ml) and hyaluronidase (1mg/ml) for 1h followed by 0.1% trypsin EDTA for 30 min at 37°C (Figure 1D). The digested cell pellet was seeded in tissue culture flask in culture medium containing Dulbecco’s modified eagle’s medium (DMEM) supplemented with 10% foetal bovine serum, 2mM L-glutamine and 1% antibiotics (Figure 1E). After reaching confluence, the cells were trypsinized with 0.25% trypsin-EDTA solution. The culture dishes were observed under an inverted phase contrast microscope (TS-100 Nikon, Japan) for cell morphology and colony formation. The isolated cells were tested for the expression of alkaline phosphatase using alkaline phosphatase staining kit (Millipore, USA), cell viability by trypan blue staining (Sigma) and the presence of MSC surface markers for CD34 and CD44 (Ab Cam, USA) using a fluorescence activated cell sorter (BD FACS, Jazz).

Results and Discussion

Many reports have been shown for the isolation and characterization of UCT derived MSCs in humans but only a few reports were observed in animals (Zucconi et al., 2010; Moshrefiet al., loccit; Sreekumar et al., loccit). The MSCs were successfully isolated and expanded in
both explant culture as well as mixed explant-enzymatic culture(Fig 1A-E). The cells started migrating from 24-48 h itself in mixed explant and enzymatic culture where as in explant culture it is after 8-10 days. In primary cultures, the cells grew more rapidly and reached 80-90% confluency on 6th day of culture (Fig 2B). The MSCs showed a varying morphology with spindle shaped, elongated, cuboidal and fibroblast like cells (Fig 2A). Confluent cells were arranged in parallel arrays. These features were observed by several studies, and they found morphology of MSCs similar to our findings (Wang et al., 2004).

The ability of the cells to form colonies is a measure of stemness of a progenitor cell activity. Presence of red reaction is an indication of strong AP activity. In the present investigation, the MSCs were isolated from WJ and examined in terms of their growth characteristics, clonogenicity and phenotypic characterization. A formal demonstration of self-renewal was provided for our isolated cells (Fig 2C). These colonies showed a strong red colour when stained with alkaline phosphatase staining kit similar to the results obtained by Moshrefi et al., (loc cit.) in goat umbilical cord matrix. The isolated MSCs expressed strong mesenchymal marker CD44, while they are negative for the hematopoietic marker CD34 (Fig 2 D, E) suggestive of UCT-WJ derived cells were one of the MSC populations similar to those derived from bone marrow and adipose tissue. Our findings are in consistent with other studies in human (Wang et al., loccit). In conclusion, the WJ derived stem cells have MSC like morphology, clonogenic, alkaline phosphatase positive and expressed MSC specific surface markers similar to bone marrow and adipose derived MSCs. It could also be inferred that whenever a fast propagation of MSCs is demanded, the enzymatic - explant culture method is best method from the UCT derived WJ tissue.

Summary
In this study, the cells with MSC phenotype were isolated from both explant and enzymatic - explant method. Both the procedures were shown to form colonies, strong alkaline phosphatase activity and the cells appeared as homogenous spindle shaped and typical fibroblast like shape...
cells. By using flow cytometry, the MSCs showed positive for CD44 and negative for CD34 surface markers. These findings are indicated that in mixed-enzymatic explant culture method, the MSCs can be isolated faster from the ovine umbilical cord wharton’s jelly.

Acknowledgement

The authors would like to thank the Department of Biotechnology, Government of India, New Delhi for their financial support for this project scheme No. BT/PR7142/MED/31/205/2012.

References


