**Introduction**

Growth of cranial bones takes place by apposition and resorption at the periosteal surfaces and by sutural growth. Normal skull development is reflected in the balance of cell proliferation, differentiation, and apoptosis in the calvarial sutures. Morphogenesis and phenotypic maintenance of cranial suture tissue are regulated by local interaction with the underlying dura. Recent studies demonstrated that dura mater regulates the suture fate by providing signaling molecules and cells that presumably undergo differentiation and migrate to the suture sites [1-2]. Abnormalities in dura-suture interactions lead to premature suture fusion clinically called craniosynostosis [3]. An important step to better understand and analyze the interplay between dura mater and associated cranial suture is to characterize dura cell phenotypes. However, few studies have been done yet. In this study we hypothesize that dura mater comprises a subpopulation of mesenchymal stem cells that can be differentiated into several mesodermal cell lineages, such as osteoblast and chondrocytes. At present study, fibroblast-like cells were isolated from rabbit dura mater and demonstrated their osteogenic and chondrogenic potentials in vitro by various histochemical and immunohistochemical approaches.

**Material and Methods**

**Cell Harvest**

Dura mater was steriley removed from 7 neonatal rabbits. The dissected tissue was finely minced, washed with phosphate-buffered saline (PBS) and digested with 0.075% collagenase type I at 37°C for 45min. The harvested cells were then plated at a density of 10^5 cells/per 100-mm petri dishes and cultured in basic medium composed of DMEM, 10% FBS and 1% antibiotics at 37°C incubator with 5% CO_2_. Culture medium was changed every third day. Upon reaching 80-90% confluence, cells were trypsinized using trypsin/EDTA (GibcoBRL, Carlsbad, CA) and collected for osteogenic and chondrogenic differentiation study.

**Osteogenic differentiation**

Cells were subcultured in 6-well plates at a density of 10^6 cell/ per well and evaluated for their differentiation potential by exposing the cells to osteoinductive medium for 6 weeks. The cells cultured in basic medium served as controls. Osteogenic differentiation medium consisted of basic medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 0.05 mM ascorbic acid-2-phosphate. On days 7, 14, 21, 28 and 42, cells from both osteoinductive and control groups were washed twice with PBS, suspended and lysized into 1% Triton-X100 solution (Sigma). The collected cells were subsequently homogenized using sonication (Dismembrator Model 100, Fisher Scientific, ). Osteogenic differentiation was assessed by ALPase activity, von Kossa and ALP staining, and osteocalcin content measurements.

**Chondrogenic differentiation**

Chondrogenic differentiation was induced using high-density “micromass” culture technique. Ten-microliter drops of cellular suspension were placed in a petri dishes and incubated for 2 hours at 37°C and 5% CO_2_. The dishes were then filled with basic medium and incubated over night. The next day chondrogenic supplements consisting of 10ng/ml TGF-beta1, 6.25 µg/ml of insulin, 6.25 µg/ml of transferrine, and 0.1 µM of dexamethasone were added to basic medium. Control group consisted of cellular pallets cultured in basic medium. On days 7, 14, 21, 28 and 42, cells from both chondrogenic and control groups were washed twice with PBS and embedded in paraffin for further histological assessment (Safranine O, H&E staining).

**DNA content and osteogenic assays**

The cell proliferation was quantitatively evaluated by genomic DNA content with fluorescent labeling DNA quantitation kit (Hoechst 33258, Bio-Rad). Alkaline phosphatase activity was measured with an ALPase Diagnostics kit (Sigma). Osteocalcin content was measured by osteocalcin ELISA kit (Quidel Co, San Diego, CA). All procedures followed manufacturers’ protocols. At each of the above time point, the plated cells were fixed with formalin and evaluated of their osteogenic differentiation potential by using ALPase and calcium deposition by von Kossa staining. Student T tests were used for statistical analyses.

**Results**

Fibroblast-like cells were isolated from rabbit dura mater tissue in basic medium (Fig. A). Upon 1-week treatment with osteogenic supplement medium, the cell morphology changing into cubic shape and higher density nodule formation was observed (Fig. B). The cells showed positive reaction to alkaline phosphatase staining (Fig. D) compared to negative reaction by control group exposed to the basic medium(Fig. C.).

High-density micromass cultures of dura-derived cells under the chondrogenic stimulation condensed into small spheroids that were visible to the naked eye as early as 48h after initial differentiation induction. The nodules that formed the micromass cultures supplemented with chondrogenic medium stained positively for Safranine O (Fig. G and H) and H&E (Fig.E and F) and at 1 and 3 weeks after initial differentiation induction. No nodules were formed under the control medium.

**Discussion**

The present study is the first report to reveal osteogenic and chondrogenic differentiation potentials of dura mater-derived cells. As specific osteogenic and chondrogenic differentiation markers of mesenchymal stem cells, increased both alkaline phosphatase activity and glycosaminoglycan content of dura-derived cells qualitatively demonstrated multifunctional characteristics of dura-derived cells. To further confirm its multiple differentiation potentials of dura-derived cells as mesenchymal stem cell, more studies including quantitative analysis are needed to differentiate dura cells into other lineage of cells, such as adipogenesis.

**References**


**Acknowledgements**

This research was supported by Whitaker Foundation Biomedical Engineering Research Grant and March of Dimes Foundation.

---

**51st Annual Meeting of the Orthopaedic Research Society**

**Poster No: 0977**