Osteogenic Construct with Potential for Treatment of Primary Cleft Palate
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Introduction
The cleft lip (CL) and palate are the most frequent craniofacial malformations in Mexico (1), and reconstruction of these defects still represents a challenge in the rehabilitation of these patients. The alveolar bone grafting is necessary in most of these patients however, this procedure is associated with high morbidity and graft tissue limited supply (2). Development of bone using a construct seeded with osteoprogenitor cells by tissue engineering procedures would allow these patients to avoid the complications of the donor area like iliac crest wound, hematoma, infection, post surgical reabsorption, etc., and it may also decrease the surgery time. The aim of this study was to create an osteoinductive scaffold as a construct with potential for treatment of cleft palate due to its osteogenic features.

Materials and Methods
Ten pediatric periosteal biopsies from the alveolar region were obtained during surgery repair (cheiloplasty) of CL, with the consent of the patients and their parents. The periostial sections were cut into small pieces and rinsed several times in PBS containing antibiotics. Tissues were cultured in vitro standard conditions in DMEM-F12 with 10% of fetal calf bovine on petri dishes as explants, until cultures were confluent. After 20-30 days, cultures were trypsinized and resuspended in culture media. Cells were seeded onto hydroxyapatite / xanthan / chitosan films and were cultured for 2 weeks in vitro conditions. Cell phenotype was studied by flow cytometry and RT-PCR before seeded onto the polymer. Constructs were analysed by immunohistochemistry, Von Kossa staining and phosphatase alkaline.

Results
We studied 10 patients ranged from 2 to 11 years. The cultures showed 100% cell viability. The isolated cells showed stem cell phenotype (CD73, CD90 and CD166 positive and CD45 negative) RUNX2, and osteopontin by RT-PCR. After being seeded on the scaffold (hydroxyapatite / xanthan / chitosan) cells showed bone matrix composed of collagen I and X (immunohistochemistry); also was mineralized (von Kossa positive, fig.1) and expressed. alkaline phosphatase. All these features are compatible with osteogenic differentiated lines.

Discussion and Conclusions
In this study, a hydroxyapatite / xanthan / chitosan with periosteal cells developed an osteogenic construct capable of forming a mineralized collagen bone matrix. This construct is able to regulate bone remodeling processes producing enzymes (alkaline phosphatase), osteoinductive proteins (osteopontin), and osteoblast differentiation factors (RUNX2). The isolated cells showed the phenotype of adult mesenchymal stem cells (positive for CD73, CD90 and CD166, and negative for CD34, CD14 and CD45). The technique of culture, expansion and differentiation of bone lodged requires 4 weeks of culture, less time than other procedures of cell differentiation. Periosteal population was composed of mesenchymal stem cells mainly. Cells differentiated into an osteogenic cells were capable of forming a mineralized collagen matrix and regulating bone remodeling process through the expression of RUNX2, osteopontin and alkaline phosphatase when were seeded onto hydroxyapatite / xanthan / chitosan polymer. These construct characteristics suggest potential for treatment of cleft palate.

References

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Disclosures
Authors have nothing to disclose.