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The innate osteogenic potential of the maxillary sinus (Schneiderian) membrane: an ectopic tissue transplant model simulating sinus lifting

S. Srouji[1,2], D. Ben-David[1], R. Lotan[1], M. Riminucci[3], E. Livne[1], P. Bianco[3]

[1]Department of Anatomy and Cell Biology, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel; [2]Department of Oral and Maxillofacial Surgery, Carmel Medical Center, Haifa, Israel; [3]Department of Experimental Medicine and Pathology, La Sapienza University, Rome, Italy

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Abstract. Maxillary sinus membrane lifting is a common procedure aimed at increasing the volume of the maxillary sinus osseous floor prior to inserting dental implants. Clinical observations of bone formation in sinus lifting procedures without grafting bone substitutes were observed, but the biological nature of bone regeneration in sinus lifting procedures is unclear. This study tested whether this osteogenic activity relies on inherent osteogenic capacity residing in the sinus membrane by simulating the *in vivo* clinical condition of sinus lifting in an animal model. Maxillary sinus membrane cells were cultured in α-MEM medium containing osteogenic supplements (ascorbic acid, dexamethasone). Cultured cells revealed alkaline phosphatase activity and mRNA expression of osteogenic markers (alkaline phosphatase, bone sialoprotein, osteocalcin and osteonectin) verifying the osteogenic potential of the cells. Fresh tissue samples demonstrated positive alkaline phosphatase enzyme activity situated along the membrane–bone interface periosteum-like layer. To simulate the *in vivo* clinical conditions, the membranes were folded to form a pocket-like structure and were transplanted subcutaneously in immunodeficient mice for 8 weeks. New bone formation was observed in the transplants indicating the innate osteogenic potential within the maxillary Schneiderian sinus membrane and its possible contribution to bone regeneration in sinus lifting procedures.

Key words: osteoprogenitors; sinus lifting; sinus membrane; alkaline phosphatase.

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Insufficient bone volume and bone quality have been regarded as common limitations for inserting dental implants in the posterior maxilla[1,7]. The most commonly used technique to reconstruct the posterior region of the maxilla is augmentation of the maxillary sinus floor, a technique introduced by Boyne & James and modified by Tatum and by Wood & Moore, in which different osteoconductive materials (bone substitutes) were placed between the host bone and the sinus membrane to allow the insertion of dental implants[4,33,36]. Recent clinical studies described a surgical technique combining

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an open controlled sinus membrane lifting procedure with immediate installation of dental implants that resulted in bone formation only by simple elevation of the maxillary Schneiderian sinus membrane (MSSM) without any adjunctive graft materials[9,10,16,17,22,26,28,32,35,36]. Case reports have described successful bone formation following cyst and tooth removal from the maxillary sinus without any grafting[18,21].

Recently, a biological insight regarding the human MSSM (hMSSM), indicated that hMSSM-derived cells have osteogenic potential[31]. Using an *in vitro* and *in vivo* study it was shown that cells isolated from the Schneiderian membrane were able to proliferate and to differentiate in culture along the osteogenic lineage. They were shown to synthesize typical osteogenic proteins and the osteogenic functionality of these cells was validated by *in vivo* bone formation in ectopic transplants in immunodeficient mice. GRUBER et al.[14] have shown that cells derived from porcine sinus-associated mucosa express STRO-1, a marker of osteoprogenitor cells, and respond to bone morphogenetic protein-6 (BMP-6) and BMP-7. Overall, these studies indicate the possible innate osteogenic potential of the Schneiderian membrane.

Histologically, the hMSSM is composed of several layers including an epithelial lining, a richly vascularized lamina propria and the deepest layer of the maxillary bone interface. This deepest layer represents an interface with the underlying bone, which is possibly equivalent to a periosteum-like structure[31]. A recent study has shown that the periosteum of the maxillary bone includes osteoprogenitor cells that can be isolated in culture and successfully transplanted *in vivo* to induce ectopic bone formation[8]. It has been reported that mechanical stimulation of a periosteum by elevation of periosteal lining resulted in massive bone formation beneath this elevation in calvaria of minipigs[20] and that similarly, periosteal distraction performed in rabbit mandible resulted in substantial bone formation underneath the mechanically elevated periosteum[6].

Based on these observations, the authors propose a possible hypothesis that bone regeneration that occurs in sinus lifting procedures is related to an osteogenic response associated with the periosteum-like membrane that lines the maxillary bone. Surgically lifting the sinus mucosa could also result in lifting of this periosteum-like membrane. This hypothesis could provide help to explain the clinical studies conducted in sinus lifting. There is no consensus on the ostoeogenic potential of hMSSM or its role in bone regeneration.

The purpose of the present study was to address this hypothesis using an *in vivo* assay to simulate the surgical condition that occurs during sinus lifting by applying an ectopic tissue transplant of the HSSM in an animal model.

Materials and methods

Protocols for the study were approved according to the ethical guidelines of the Carmel Medical Center, Haifa, Israel. The hMSSM samples were obtained with informed consent from patients, aged 18–25 years ($n = 5$), who suffered from posterior or total maxillary excess, undergoing posterior or total maxillary superior impaction for orthognathic surgery. Smokers or patients with skeletal disorders and syndromatic diseases were excluded. Bone segments were removed from the posterior maxilla (lateral wall of the maxillary sinus) prior to the impaction. The hMSSM in the medial side of the segment was separated and collected during surgery and placed in phosphate buffered saline (PBS) supplemented with antibiotics. The samples were used for enzyme histochemical analysis, to establish an *in vitro* culture of hMSSM-derived cells and for *in vivo* transplantation in immunocompromised mice.

Cell isolation from hMSSM

For isolation of cells, samples of the hMSSM were extensively rinsed with PBS solution supplemented with antibiotics, and cut into small pieces. The tissue fragments were incubated with dispase (Sigma–Aldrich, St. Louis, MO, USA, 37 °C, 1 h) to separate the epithelial lining from the membrane. The epithelial layer was separated and discarded. The remaining tissue fragments were incubated in collagenase containing solution (Collagenase type II, Sigma–Aldrich, USA) 150 U/ml + 3 mM $CaCl_2$ in Hank's balanced salt solution (HBSS) for 2 h with constant rotation. The resulting cells were counted and 5×10^5 cells were plated in 10 cm-diameter tissue culture dishes with α-MEM medium containing 10% foetal calf serum (FCS), 2 mM L-glutamine, Pen-Strep (both 100 U/ml), (Biological Industries, Beit Haemek, Israel). To induce osteogenic differentiation in culture the cells were passaged and P1 cultures were cultured for an additional 14 days in α-MEM medium containing 10% FCS, 2 mM L-glutamine, Pen-Strep (both 100 U/ml), 100 g/ml ascorbic acid and 10^{-8} M dexamethasone (induction medium).

Alkaline phosphatase enzyme histochemistry in fresh hMSSM

To demonstrate alkaline phosphatase (ALP) activity, tissue samples were fixed in cold acetone for 1 h, embedded in paraffin, and 6 μm thick sections were reacted with Gomori's calcium cobalt method[12], using β-glycerophosphate as a substrate (Sigma–Aldrich, USA) and stained with haematoxylin-eosin (H-E) for general histology.

ALP enzyme activity in cell culture

hMSSM-derived cells grown in control and in inductive medium for 3 weeks in culture, were washed twice with PBS, fixed with 4% formaldehyde in phosphate buffer, pH 7.4, and reacted for ALP using Naphthol AS phosphate as substrate and Fast Blue BB as coupler (Sigma–Aldrich, USA). Naphthol AS phosphate was dissolved in *N-N′* dimethylformamide (30 mg in 0.5 ml) and added to a 0.1% solution of Fast Blue BB salt (Sigma–Aldrich, USA) in 0.1% boric acid/sodium tetraborate buffer, pH 9. Cultures were incubated in the ALP substrate solution for 20 min at 37 °C.

ALP enzyme activity in cell culture

Additional MSSM-derived cells were grown in control medium and in inductive medium for a maximum of 18 days in 24-well culture plates. At predetermined times (4, 8, 12, 19 days) cells were washed twice with PBS, lysed with cold lysis buffer (1 mM $MgCl_2$, 0.5% Triton X100 in alkaline buffer solution) and incubated on ice for 1 h, another equivalent portion of lysis buffer was added to the sample. Cell lysates were mixed with phosphatase substrate solution (4 mg/ml *p*-nitrophenol phosphate (*p*-NPP) in alkaline buffer Tris–HCl, pH 9.0) for 10 min at 37 °C and then returned to ice, the reaction was stopped with ethylene diamine tetra-acetic acid (EDTA)–NaOH stop solution. The samples were transferred to a 96-well plate and absorbance was read at 404 nm using an Elisa plate reader. The results were expressed as nmol *p*-NP/ml/min and normalized to protein content as measured by the BRADFORD[5] method in corresponding wells.

Reverse transcription polymerase chain reaction

Total RNA was prepared from cells (P_1; induction medium) collected from