Tissue Engineering in Dentistry: isolation and characterization of osteogenic stem cells from human jaws periosteum

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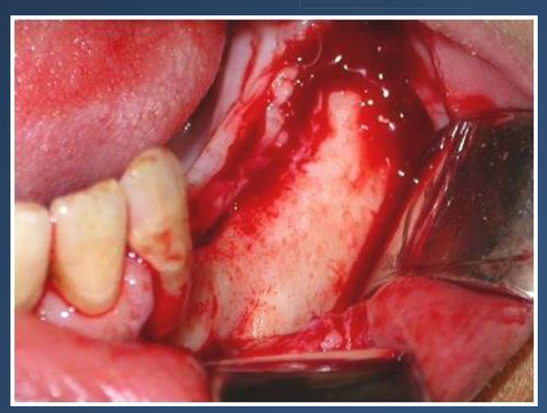
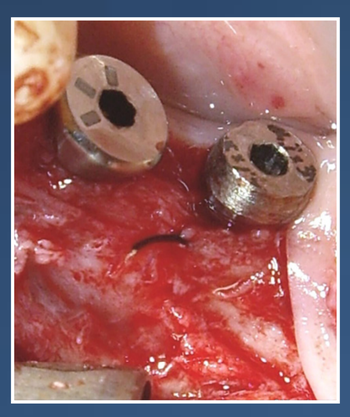
Aim of our study was to identify sources of stem cells in human adult oral cavity, which can be of interest in the dental practice. In this study we focused on periosteal cells isolated from periosteum of human maxilla or mandibula. Periosteum (5 mm x 5 mm square) from human maxilla or mandibula was taken during normal surgery procedures in the oral cavity (e.g. implant placement, periodontal/bone regeneration), for a total of 30 specimens and patients (aged 32 to 68) and an average weight of 0.96 grams. The tissue was gently minced, then digested enzymatically in a Collagenase type II 0.25% solution for 90 mins at 37° C, then plated. Periosteal stem cells (PSCs) were cultivated in Coon's F12 with 10% FBS added with 2 mM Gln, penicillin 100 U/ml and streptomycin 0.1 mg/ml. Medium was changed every 4 days. We made some in vitro studies to characterize this cell population (Alkaline Phosphatase and Collagen I, and RT-PCR) and also took advantage of the immunodeficient mouse model to preliminary test PSCs' osteogenicity in vivo: they were loaded onto a 4 mm cubic porous ceramic (hydroxiapatite) scaffold and implanted subcutaneously; animals were sacrificed at 2 and 4 months after surgery. After 10 to 15 days the PSCs were clearly visible in the culture dishes. Phenotypic characterization by immunohistochemical analysis showed that PSCs were positive for Collagen type I and ALP activity. By RT-PCR investigation we observed that PSCs expressed Runx2 (cbfa-1), BSP and Osteocalcin. This means that this cell population expresses both early and late bone markers. The histological analysis of the scaffolds implanted in the immunodeficient mice showed the formation of a good quantity of lamellar bone inside, both at 2 and 4 months after the implant. Therefore PSCs – isolated during ordinary surgery procedure in the everyday dental practice in our office – should be considered with great attention in the future clinical procedures, as a valid adult stem cells source. Our in vitro studies showed a strong osteogenic commitment of this population, and the immunodeficient mouse model showed PSCs' capacity to form bone in vivo. Further investigations are needed, of course, but this seems to be quite a good pathway.

ISOLATION & CULTURE

Harvesting

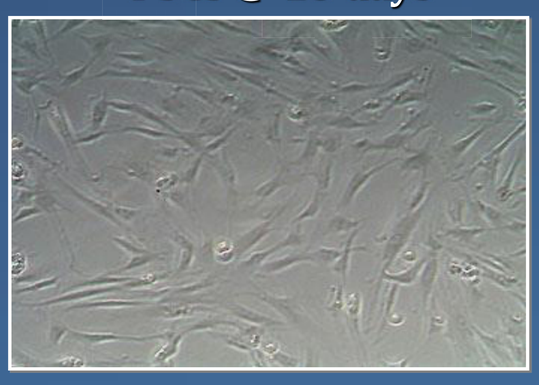




Digesting

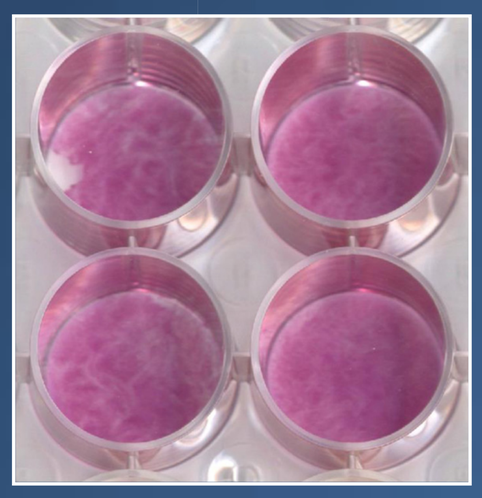
Plating

PSCs @ 15 days



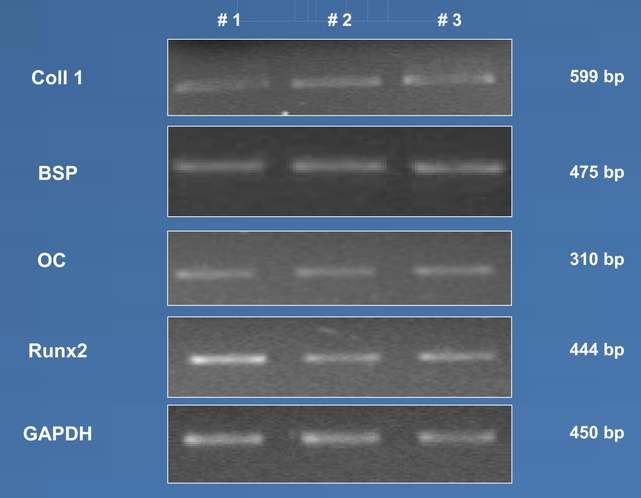
IN VITRO CHARACTERIZATION

ALP

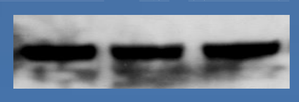


Collagen I

RT - PCR



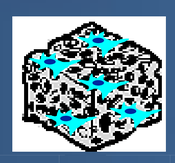
Western Blot (periostin)



IN VIVO CHARACTERIZATION

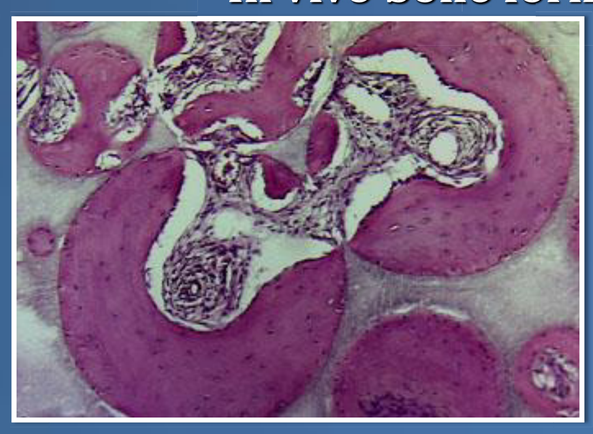
Detaching

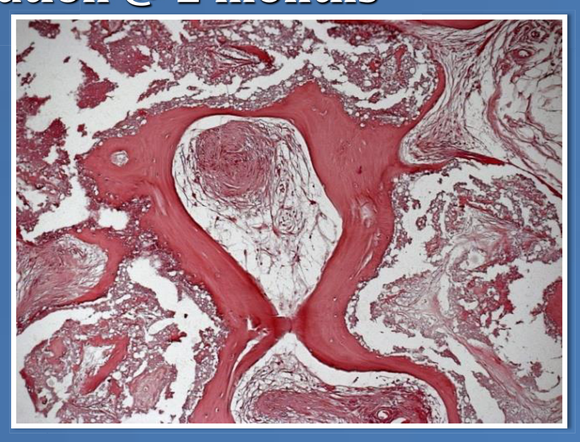
ID mouse model

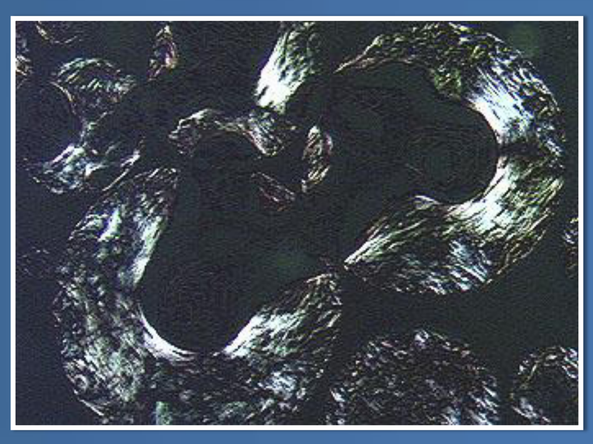


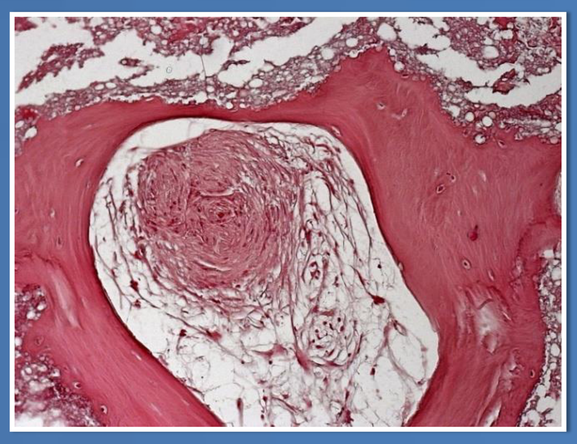
PSCs loading onto
4 x 4 mm HA cube

In vivo bone formation @ 2 months









Bone @ 4 months