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# Isolation of human osteoblasts and in vitro amplification for tissue engineering and subsequent bone repair

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November 1999
4th European Skull Base Society Congress
3. Kongress der deutschen Gesellschaft für Schädelbasischirurgie
Nürnberg

## Objective

Traumatic injuries, cancer treatment and congenital disorders with abnormal bone shape or segmental bone loss requires replacement of missing bone. This may be accomplished by implantation of bone substitute material or by surgical transfer of natural tissue from an uninjured location elsewhere in the body. However, these procedures are limited due to different disadvantages. One strategy to overcome these problems is to develop living substitutes based on tissue engineering. As first step we have investigated the possibility to establish osteoblast cultures from facial bone.

## Material and Methods

**Cell Culture:**
From 5 patients small cortico-cancellous bone chips of the maxilla were gained during routine osteotomies in oral and maxillofacial surgery and divided further in 1 x 1 mm explants. These explants were seeded on the floor of the culture dishes and cultured in Dulbecco's Modified Eagles Medium with bovine serum. The primary cultures were further subcultured, using 0.1% trypsine in PBS.

**Alkaline Phosphatase (AP) Histochemistry:**
To determine the alkaline phosphatase activity in the cell cultures morphologically, the cultures were air dried and after fixation in formol and absolute alcohol incubated with the substrate mixture fastblue salt, sodium nitrite solution, alkaline naphthol AS BI solution in the dark for 20 min. The specimen were counterstained with May Grünwald Giemsa.

**Collagen (Coll) I Immunohistochemistry:**
After fixation of the specimen in ethanol 70%, collagen type I was demonstrated by immunhistochemistry. The collagen type I antibody (Sigma, Munich, Germany) (Dilution 1:1000) and Vecta Stain was used according to the standard protocol.

**Histomorphometric Assessment:**
The amount of AP and Coll positive cells was assessed planimetrically. From each culture 5 visual fields were analysed using Zeiss Axiolab microscope and the Analysis 2.1 soft ware (Softimaging Software GmbH, Münster, Germany). The amount of labelled cells was expressed as percentage of cells counted in total.

## Results

After 14 days of culture the first cells grew out of the bone explants, after another 2-3 weeks the floor of the culture flask was covered by a subconfluent monolayer. There were cells of multipolar configuration and spindle shape visible. Both types of cell morphology stained for AP and collagen I (compare Fig. 1 and 2)

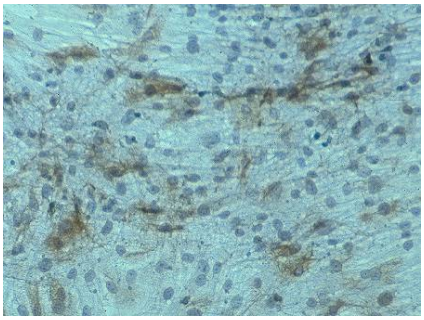

Fig 1:
Histochemistry of AP in osteoblast cultures from the maxilla. Blue / purple staining of AP positive cells (x300).

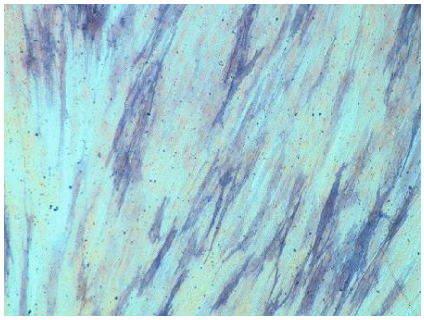

Fig 2:
Immunohistochemistry of collagen I in osteo-blast cultures from the maxilla (x 150).

During subculturing procedures, the period to establish first and secondary passages was 14 days, further subculturing resulted in a further shortened culture period of only 5 to 7 days.

The morphometric assessment of the AP and Coll positive cells over the culture periods showed a maximal expression for both markers in the second passage. In the second passage in average 72 % of all cells stained for AP whereas 25 % resp. 42 % of all cells expressed AP in the first resp. in the third passage (Fig. 3).

We observed considerable variation between the osteoblast cultures of different human individuals.

The assessment of collagen I similarly showed a maximum of positive cells in the second subculture (Fig. 4).

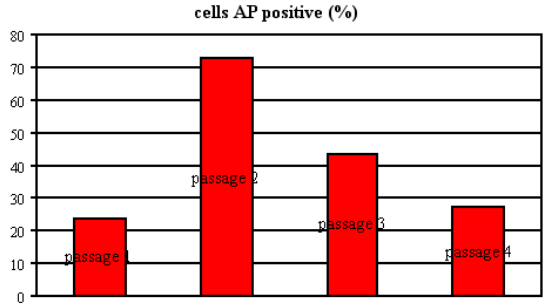


Fig 3:
Histomorphometric assessment of AP positive osteoblast cells in 4 passages of culture. There is a maximal expression in the second passage.

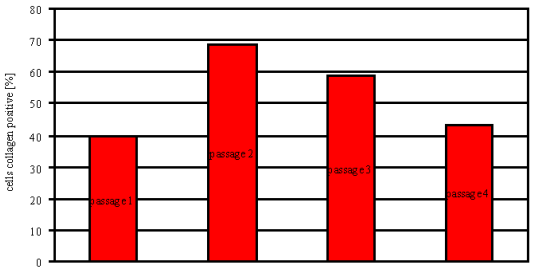


Fig 4:
Histomorphometric assessment of collagen I positive osteoblast cells in 4 passages of culture. There is a maximal expression in the second passage.

## Discussion and Conclusions

From cortico-cancellous bone chips of the maxilla cultures of human osteoblast like cells can be established. The amplification of these cells in subculture is easy to facilitate. A maximal expression of osteoblast differentiation markers like alkaline phosphatase and collagen I could be detected in the second and third passage. The demonstration of culturing sufficient differentiated osteoblast material originating from the human maxilla is a crucial step in respect to tissue engineering of bone which will find its application in cranio-maxillofacial surgery.

*This Poster was submitted on 05/11/2001 by Ronald Schimming MD, DMD.*

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**Poster Faksimile:**

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G. Lauer[1], R. Schimming[2]

Departments of Oral and Maxillofacial Surgery, University of Dresden[1] and Freiburg[2], Germany

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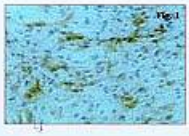

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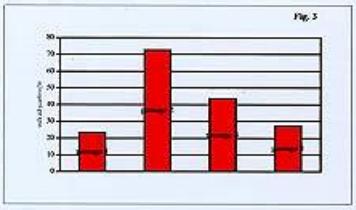

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