Ilara R. Zerbo Gert L. de Lange Manon Joldersma Antonius L.J.J. Bronckers Elisabeth H. Burger

# Fate of monocortical bone blocks grafted in the human maxilla: a histological and histomorphometric study

### Authors' affiliations:

Ilara R. Zerbo, Gert L. de Lange, Manon Joldersma, Antonius L.J.J. Bronckers, Elisabeth H. Burger, Department of Oral Cell Biology, ACTA, Amsterdam, the Netherlands

### Correspondence to:

Ilara R. Zerbo Oral Cell Biology, ACTA, Vrije Universiteit, Van der Boechorststraat 7 1081 BT Amsterdam the Netherlands Tel: + 31 20 444 8373 Fax: + 31 20 444 8683 e-mail: IR.Zerbo.Ocb.ACTA@med.vu.nl

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Abstract: Local bone defects in the anterior maxilla are commonly grafted with monocortical blocks of autologous bone in order to restore the defect site prior to the placement of dental implants. Increasing evidence suggests that osteocytes are involved in the control of bone remodelling and thus may be important for optimalisation of bone structure around implants, and thus for implant osseointegration. However, it is not well known whether osteocytes will survive when bone blocks are grafted into defects. We grafted 19 patients with monocortical bone blocks derived from the symphysis, to the defect site in the maxillary alveolar process. The bone grafts were left to heal for times varying from 2.5 to 7 months. During implant installation, bone biopsies were removed using a trephine burr, and processed for hard tissue histology. Bone histology and histomorphometry were then carried out in order to gain insight into the density, viability and remodelling of the graft. Clinically, all the bone grafts were successful, with no implant failures, and little resorption was seen. Histologically, bone volume expressed as percentage of tissue volume at the implant site varied from 27% to 57% with an overall average of 41%. Bone fields with empty osteocyte lacunae were observed and measured. The amount of this so-called nonvital bone (NVB) varied between 1% and 34% of the total tissue volume. The amount of NVB decreased significantly with the time of healing. The data suggest that the majority of the osteocytes of the monocortical bone do not survive grafting. The results indicate that the NVB is progressively remodelled into new vital bone 7 months after grafting.

Bone defects in the human maxilla are a common finding. They are mostly derived from premature loss of a tooth due to periapical infection following decay or trauma. In order to restore this situation prior to implantation, a bone augmenting procedure has to be carried out. The use of autologous bone has been accepted for a considerable time to be the most predictable method of grafting bone defects (Listrom & Symington 1988; Jensen & Sindet-Pedersen 1991; Block & Kent 1997). The bone may be harvested from a variety of sites, intraorally, from the retromolar,

ramus or the symphysis (Misch 1997; Jensen & Sindet-Pedersen 1991; Widmark et al. 1997; Hunt & Jovanovic 1999; Montazem et al. 2000) or alternatively from extraoral sites such as iliac bone, calvarium or tibia (Block & Kent 1997). The symphysis is a well-accepted donor site for grafting since it is easily accessible, contains adequate amounts of bone and can be obtained without aesthetic consequence (Devlin et al. 1994; Misch 1997). Grafts from the symphysis show less resorption, when compared to those from the crista iliaca (Koole 1994; Lundgren et al. 1996). Goldberg & Stevenson (1987) described that a bone graft has two main functions: to be a source of osteogenic cells and a mechanical support. A bone block is preferred for this type of augmentation, since it provides both the source of cells and a rigid structure. Blocks are less likely to fail due to micro-movement during the ingrowth of a new bone, which could be the case when bone chips are used. The bone that can be grafted from this area is easily shaped to fit the defect site and can be stabilised simply with screws. Although this treatment is well accepted in clinical practice, there are a number of unanswered questions about the biological process taking place in the bone graft. We wanted to know whether bone tissue cells, particularly the osteocytes that are completely entombed by mineralised bone, survive transplantation or die due to the transient lack of vascularisation. Vitality of osteocytes may be important for bone remodelling around implants, since these cells are currently believed to be involved in bone turnover by secreting signalling factors. These factors communicate with the bone surface, which control the activity of bone surface cells such as osteoblasts, osteoclasts and bone lining cells (e.g. Smith & Burger 2002; Zhao et al. 2002). It was also of interest to know the timing of the remodelling process in order to understand the biological process so that the clinical protocol could be adjusted accordingly.

Although studies have been conducted to assess the results of bone grafts from the human mandibular symphysis (Jensen & Sindet-Pedersen 1991; Misch et al. 1992; Widmark et al. 1997; Montazem et al. 2000), few studies have examined the occurrence and fate of osteocyte survival in bone blocks grafted into the human jaw using quantitative histomorphometry (Blomqvist et al. 1998).

This study aimed to gain insight into the fate of bone grafts into the human jaw with emphasis on the survival of the osteocytes and graft vitality.

# Material and Methods Patients

Nineteen patients (15 men and 4 women) of ages ranging from 17 to 57 years were included in the study. Patients selected had a severe defect in the anterior maxilla, with complete loss of the buccal plate that required regeneration with a bone block, of such dimension that the implant (and thus biopsy) would be placed in the grafted bone only. Patients had a variety of causes for the loss of their teeth, ranging from trauma to periodontal disease. All 19 patients were healthy and one of them (patient no. 1, see Table 1) was a smoker.

A clinical and radiographical investigation was carried out and the treatment fully explained. Patients were scheduled first for the bone augmentation followed by a period of healing and eventually a second operation for biopsy taking and implant placement. All patients were willing to donate the tissue to be removed during implant surgery for histological examination. Eight patients gave informed consent to use tetracycline for bone labelling. The bone labelling protocol consisted of one tablet of 200 mg tetracycline (tetracycline hydrochloride, Katwijk Farma, Leiden, the Netherlands) three times a day for 2 consecutive days, followed by 2 weeks without tablets and then a further 2-day course. Labelling occurred between 1 and 2 months prior to biopsy retrieval.

### Clinical procedure

The bone augmentation procedure was carried out under local anaesthesia and in sterile conditions. The mental nerves were bilaterally marked after carefully studying the patients' radiographs and palpation of the mental foramina. A partial thickness incision was made 10 mm apically of the margin between attached and loose gingiva of the lower incisors. The partial thickness incision was then extended apically until approximately 0.5–1.0 cm below the apex of the lower incisors where a full-thickness

Table 1. Results of histomorphometry means

Pat.	Age (years)	Sex	Time (months)	BV (%)	NVB (%)	OV (%)	RS (%)	MAR (µm/day)
1	31	М	7	50.9	5.1	0.8	0.2	
2	17	F	4	55.3	18.4	0.8	0.2	1.3
3	17	М	6	27.3	0.7	0.3	0.4	
4	52	F	5	30.1	2.6	0.7	1.4	
5	32	М	6	57.1	2.1	1.6	1.8	
6	36	М	4	42.5	3.3	1.8	1.7	
7	30	М	3	49.4	10.1	1.1	0.5	
8	33	F	4	46.7	4.7	1.6	0.6	
9	59	М	3	37.7	27.7	0.5	1.5	
10	49	М	4	36.7	4.8	1.0	1.2	
11	18	М	3	30.8	3.1	0.9	0.8	1.6
12	22	М	4	29.4	14.7	1.2	1.2	1.7
13	25	М	2.5	39.3	34.2	0.9	0.6	
14	55	М	3	41.2	4.1	1.4	0.5	0.7
15	37	М	4	30.1	20.1	0.3	0.2	1.5
16	17	F	3.5	54.0	22.4	0.9	1.1	1.4
17	18	М	5	45.3	1.1	1.6	1.2	
18	34	М	3	37.8	18.2	0.8	1.2	2.3
19	52	М	6	38.3	13.1	0.6	0.4	
Mean	33		4.2	41.0	11.1	1.0	0.9	1.5

flap was then made. The symphyseal bone was fully visualised and a bone block was cut with ample irrigation with a thin stainless-steel bur. The two holes for fixation in the defect site were also prepared, while the block was in situ and a countersunk provided so that the head of the screw was level with the graft surface. The block was then lifted from its site by gentle easing with an osteotome and hammer. The donor site was filled with bioactive glass particles (Biogran<sup>®</sup>, 3i Implant Innovations Inc., Palm Beach Gardens, FL, USA) and closed in two stages, a deep periosteal layer and a superficial mucosal layer, in order to avoid postoperative bleeding and any wound dehiscence as a result of swelling.

The defect site was prepared by opening an adequate full thickness flap. The defect site was thoroughly cleaned in order to remove inflamed or infected tissue. The bone block was reshaped to fit the defect site and then fixed to it via two screws (Fig. 1a,b). The graft was then covered with a resorbable membrane. Usually, the membrane chosen was Biogide<sup>®</sup> (Geistlich, Wohlhazen, Switzerland), but in two cases, a nonresorbable membrane was used: in patients no. 1 and 4, a PTFE membrane (Gore, Flagstaf, AZ, USA) was used. The flap was then closed over the bone graft in order to maintain the blood supply and sutured carefully in order to completely seal the wound. The temporary prosthesis was altered to avoid loading of the graft. The sutures were removed 1 week later. Implants were placed 2.5-7 months later. During osseointegration of the implants, loading was again avoided by altering the temporary prosthesis.

### **Biopsy procedure**

At the time of implant placement, the biopsies were taken exclusively from the grafted bone area (Fig. 1c). An initial preparation for the implant was made with a hollow trephine burr (ITI- Straumann, Switzerland) of 2.8-mm outer diameter and with copious



*Fig. 1.* Cross-section of maxilla showing the defect, position of the graft and the procedure of performing the biopsy. (a) A defect has developed on the buccal aspect leaving insufficient bone for placement of an implant. (b) The defect is augmented with a monocortical bone block from the symphysis and left to heal after immobilisation with two screws. (c) The screws have been removed after healing and the biopsy is removed from the original graft with a trephine burr.

irrigation. The biopsy was then the material from the inner core of the trephine burr. In this manner, a biopsy of approximately 2.5mm diameter and 8–10 mm long was obtained (see Fig. 2a, b). The biopsy was carefully pushed out of the trephine burr by using an especially designed instrument provided by the manufacturer. The apical part of the biopsy was labelled by black Indian ink, for orientation during histology.

### Histology

All biopsies were immediately fixed in 4% formaldehyde solution in 0.1 M phosphate buffer (pH 7.3) at 4°C for 24 h (see also Tadjoedin et al. 2000). They were then rinsed three times with 0.1 M phosphate buffer and finally stored at 70% ethanol at 4°C, until ready to be embedded. All 19 biopsies were cold embedded in methylmethacrylate with 20% plastoid. Undecalcified, 5-µm thick sections were made along the axis of the biopsy using a Jung K microtome. Four groups of five consecutive sections were cut, with a distance of 225 µm between each group. The first and second sections from each group were stained with Goldner's trichrome method (Romeis 1989). This procedure stains osteoid and other demineralised bone matrix red, mineralised bone matrix green and cytoplasm blue /black. The third section of each group was used to identify osteoclasts using tartrate-resistant acid phosphatase (TRAP) (Van de Wijngaert & Burger 1986) stain, modified by the addition of 3 mM polyvinyl alcohol (Sigma P 8136,



*Fig.* 2. (a) Monocortical bone grafted into the maxilla on re-opening after 6 months healing (see dotted line for outline). Note the biopsy cut with a hollow trephine burr, *in situ*. In this case, the biopsy did not initially come out with the trephine, but stayed in the maxilla and was later carefully removed. (b) The biopsy removed from the augmented site. Bar = 0.5 cm.

Mw = 30-70 kDa) to the incubation medium and counterstained with light green. TRAP-positive cells stain red, while mineralised bone matrix and connective tissue stain green. Finally, one section of each group was left unstained for fluorescence microscopy in order to see the tetracycline labels, while the fifth section served as reserve.

### Histomorphometry

A Leica DM RA microscope connected to a computer using an electronic stage table and a Leica DC 200 digital camera were used for histomorphometrical measurements. The computer software used was the Leica QWin<sup>©</sup> (Leica Microsystems Image Solutions, Rijswijk, the Netherlands) to process and measure the digitised image. This software allows selection of parts of an image by setting a threshold to the colour of the pixels, which can then be measured. Four evenly spaced sections (one from each group) from each biopsy were used for the measurements. In this manner, a clear view throughout the biopsy could be observed and the measurement became more accurate, since it accounted for variation throughout different depths in the biopsy. All measurements were carried out at  $\times$  200 magnification in order to allow clear distinction between empty and full osteocyte lacunae. The sections stained with Goldner's trichrome were used for the measurements of total bone volume (BV), nonvital bone (NVB) volume and osteoid volume (OV). These were measured by consecutive fields until the whole section had been quantified. The number fields varied between 40 till over 80 fields, depending on the length of the biopsy. The total (absolute) BV was calculated as the amount of mineralised (green) bone tissue as a percentage of the total tissue volume (TV), (thus  $BV/TV \times 100\%$ ), according to Parfitt et al. (1987). The mineralised bone tissue that contained areas of empty osteocyte lacunae was defi-ned for this study as the NVB. NVB was expressed as percentage of the TV (NVB/ TV × 100 %). The measurement of this volume was made semiautomatically at the same time as the BV measurement, by out-lining the area of empty osteocytes lacunae.

The absolute OV was the total area of osteoid as a percentage of the total TV (OV/  $TV \times 100\%$ ). TRAP- stained sections, con-

secutive to the Goldner-stained sections, were used to identify areas of TRAP positive cells and Howship's lacunae and to measure the resorption surfaces (RS). The RS was defined as the scalloped surface and was expressed as a percentage of the total bone surface (BS) (RS/ BS × 100%).

Where applicable, fluorescent (tetracycline) labels were measured in unstained sections using an excitation wavelength of 354-425 nm and emission wavelength of 470 nm. The average distance between two labels was calculated by measuring the total area between the two bands and dividing it by their average length. Ten consecutive double labels were measured, when possible from the same section, and the average calculated. The mineralisation apposition rate (MAR) is the average distance between fluorescent bone labels divided by the number of days between the two courses of the tetracycline and is expressed as µm/day.

### Statistical analysis

All data were analysed using SPSS for Windows 9.0. The nonparametric, one-sided Spearman's correlation test was used to examine the correlation between healing time and histomorphometry volume. The data are presented as means and standard deviations. Significance was accepted when P < 0.05.

# Results

# Clinical

All 19 grafts were successfully integrated, providing sufficient bone for implant installation. They showed no resorption around the head of the fixation screws. One patient (no. 4), in whom a nonresorbable membrane was used, experienced a small membrane exposure. The membrane was then removed and no further symptoms were noticed. One patient (no. 7) had an allergic reaction to the suturing material (Vicryl), which once removed, resolved spontaneously. One patient had paraesthesia of the upper lip, which recovered only partly. All 19 implants were eventually restored with a cast abutment and a cemented crown. There have been up to the time of writing this paper, 12 months or more after fitting of the prosthetic work, no further complications.

### Histology

All sections contained varying amounts of vital and NVB tissue, compact osteonic bone and trabecular bone. The amounts of vital and NVB varied considerably between individuals. Bone classed as nonvital (with fields of empty osteocyte lacunae) was predominantly of the lamellar type and was in contact with, or completely surrounded by vital bone (Fig. 3a). The vital bone was composed of both lamellar and woven bone. Using polarised light microscopy, the margin between the vital and the NVB coincided with an abrupt change in the orientation of the lamellae (Fig. 3b). Very little qualitative difference could be found between biopsies of different healing times by description of the histology alone.

The amount of osteoid also varied between biopsies (see Table I). Osteoid formation was particularly prominent in highly vascularised tissue. New bone was also formed within NVB around the Haversian canals (Fig. 4).

All the tissues were free of inflammatory cells. Bone marrow was strongly vascularised and contained fat cells.

Resorption sites could be easily identified in the TRAP-stained sections, which identified the location of osteoclasts. The resorption sites were scattered and occurred predominantly in the NVB, although no specific measurements were carried out in order to quantify this.

The dynamics of lamellar bone formation were followed by tetracycline labelling, given 2 months before biopsy retrieval. Of the eight patients who received tetracycline, one could not be measured because no discrete fluorescent bands were found, but rather a diffuse label. This resulted from the patient not following the labelling protocol accurately. Of the seven remaining patients, the double labels varied in length from short to long labels. The overall distribution of labels was similar in all cases. The NVB contained no tetracycline labelling.

### Histomorphometry

The absolute total bone volume (BV) (% of the total tissue volume) ranged from 27%to 57% with an overall average of 41%. The percentage of NVB varied from under



B



*Fig.* 3. (a) Nonvital bone (within dotted line) surrounded by vital bone. Note new bone formation (O = osteoid) taking place, as indicated by black arrows. M = marrow, Ocy = osteocytes, EL = empty lacunae. Goldner's trichrome staining. Magnification × 200. (b) Polarised light microscopy of the same section as in (a). Areas of bone with empty osteocyte lacunae (within dotted line) and abrupt changes in the orientation of lamellae (white arrows) were considered to be grafted bone that had become nonvital after grafting. Magnification × 200.

1% to 34% with an overall average of 11% of total tissue volume. The average vital bone volume between all patients was 30%. The volume of vital and NVB of all the patients and the time of healing is presented in Fig. 5 and in Table 1. The relative NVB volume (NVB as % of total bone volume) varied from 87% (in patient no.13) to 2.5% (in patient no. 3). The NVB significantly decreased with increasing time of healing (r = -0.44, P < 0.05) (Fig. 6).

The OV, expressed as a percentage of the TV (OV/TV  $\times$  100%) ranged from 0.3% to 1.8% (Table 1). The mean OV throughout the patients was 1.0 ± 0.5%. No correlation

was found between the time of healing and the amount of osteoid (data not shown).

The resorption surface varied among patients from 0.2% to 1.8% of the BS. The average resorption was  $0.9 \pm 0.5\%$  of the BS. (No correlation was found between the resorption surface and the time of healing (data not shown)). Finally, the MARs of seven patients are summarised in Table 1. The MAR results varied from 0.8 to  $2.3 \mu$ m/day. The overall average MAR of all patients was  $1.5 \pm 0.4 \mu$ m/day. All patients who received the tetracycline bone labelling had a short healing period (between 3 and 4 months).

## Discussion

Our clinical findings show that 2.5-3 months after grafting, the grafted bone had integrated with the original maxillary bone and was stable enough to be implanted without clinical complications. Histologically, however, at this time, the grafted bone contained substantial amounts of NVB. It is not clear as to how long osteocytes survive once separated from their blood supply. Ellegaard et al. (1975) reported that I week after grafting of fresh cancellous jaw bone into furcation defects of monkey molars, most osteocyte lacunae were devoid of cells. A histological study in human 3-5 cm-sized rib bone fragments grafted submuscularly and retrieved 1 week later showed that 'more than 50% of the osteocytes retained their basophilic staining' (Chugh et al. 1998), implying that almost 50% of the osteocytes did not survive. In contrast, rat osteocytes maintained a normal morphology after grafting autologous fresh ribs into the mandible and examined at days 5-84 (Kamijou et al. 1994). More recent developments in orthopaedic and maxillofacial surgery have furthermore shown that vascularised grafts perform better than similar free grafts (Goldberg et al. 1987; Moran & Wood 1993). Berggren et al. (1982) showed, in a study in dogs, that the osteocytes and osteoblasts can survive up to 25 h of ischaemia if the grafts are stored in a cold culture solution followed by microvascular anastomoses. The survival of the osteocytes may therefore be partly explained by the revascularisation of the grafts. Our findings agree with the data of Ellegaard et al. (1975) and Chugh et al. (1998), illustrating that after disruption of the blood vessels many osteocytes in free, human chin bone blocks do not survive grafting. It has been stated that dead bone may be prone to fracture (Goldberg et al. 1987). From a clinical point of view, it would seem important to wait for NVB to be replaced by vital bone prior to implant loading, since vital bone has better mechanical characteristics (Goldberg et al. 1987). In this respect, the rate by which the NVB is resorbed by osteoclast activity followed by bone formation is an important factor for osseointegration of implants. Recent data suggest that osteocytes may be involved in recruiting osteoclasts or modulating osteoclast

activity by secreting signalling factors, such as nitrogen oxide, prostaglandins (Klein-Nulend et al. 1995), osteoprotegerin, M-CSF and RANKL (Zhao et al. 2002) or factors related to the apoptosis pathway (Bronckers et al. 1996, Verborgt et al. 2000) induced by ischaemia (Kikuyama et al. 2002). The vitality of osteocytes in the graft may therefore be one factor to account for in remodelling of the bone graft. It is reported by some authors that NVB is resorbed less easily by osteoclasts than vital bone. Kamijou et al. (1994) showed that rat rib bones, devitalised by freezing and grafted into the mandibles did not exhibit resorption, even after 84 days while in the control ribs, where the osteocytes remained vital, the majority of the rib bone was replaced by new bone, as early as 14 days. In contrast, other authors reported that after devitalisation of bone (by boiling or freezing bone fragments prior to grafting), the resorption was not affected greatly compared with resorption of vital bone (Ellegaard et al. 1975; Kingsmill et al. 1999). This is in agreement with the present study. We found a gradual decrease of the amount of NVB (by osteoclastic activity) with increasing time of healing. Our results also suggest that the NVB is fully replaced by new vital bone in approximately 7 months (see Fig. 6).

In contrast to our findings, Blomqvist et al. (1998) reported that I month after grafting iliac crest-derived bone into the human sinus floor, 75–100% of the osteocytes were vital and suggested that this was due to survival of the osteocytes. Their histological figures, however, show that a large number of these vital osteocytes were located in woven bone, suggesting that they were looking at bone that had formed since grafting (possibly from surviving osteoblasts and osteogenic cells) rather than true osteocyte survivals.

There is increasing evidence that osteocytes are mechanosensitive (Klein-Nulend et al. 1995) and as such play a crucial role in adaptive bone remodelling (Smit & Burger 2002). At the early stages of graft healing, osteocytes are still sparse in the grafted bone, since a large percentage of the bone is nonvital. It is important to consider how much vital bone is present at the time of loading of an implant. If the implant is allowed to osseointegrate prior to loading, during that time, remodelling of NVB can



*Fig 4.* Vital bone containing osteocytes (arrows) in the inner core of an osteon, surrounded by nonvital bone (NVB), suggesting that the NVB bone was recolonised by blood vessels (BV) and osteogenic cells via the Haversian canal. Goldner's trichrome staining. Magnification  $\times$  400.



Fig. 5. Vital and nonvital bone as percentage of total tissue volume.



Fig. 6. Nonvital bone as percentage of total bone volume relative with time.

take place. At the time of loading the bone will be predominantly vital. Mechanical loading of this bone may then stimulate bone adaptation further by promoting bone deposition and bone resorption. In short, the present study suggests that osteocytes in monocortical bone blocks of the human chin are for the greater part unable to survive grafting. However, the remodelling that follows grafting is relatively quick and free of complications. Within approximately 7 months after grafting the bone is fully remodelled, vital and in principle able to adapt fully to the functional loads it must endure.

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### Résumé

Les lésions osseuses locales dans le maxillaire antérieur sont souvent greffées avec des blocs monocorticaux d'os autogène afin de restaurer le site avant le placement d'implants. Il semble de plus en plus évident que les ostéocytes sont induits dans le contrôle du remodelage osseux et pourraient donc être importants pour optimiser la structure osseuse autour des implants et donc l'ostéoïntégration implantaire. Cependant le taux de survie des ostéocytes lorsque les blocs osseux sont greffés dans les lésions n'est pas suffisament connu. Dix-neuf patients ont été greffés avec des blocs osseux monocorticaux provenant de la symphyse dans le site de la lésion au niveau des alvéoles maxillaires. Les greffons osseux sont restés in situ durant des périodes de 2,5 à 7 mois. Pendant l'insertion des implants des biopsies osseuses ont été prélevées avec un trépan et analysées par histologie. L'histologie osseuse et l'histomorphométrie ont été effectuées afin d'analyser la densité, la viabilité et le remodelage osseux. Cliniquement tous les greffons osseux ont été effectués avec succès sans aucun échec implantaire et peu de résorption. Histologiquement,

le volume osseux exprimé en tant que pourcentage du volume tissulaire au site implantaire variait de 27 à 57 % avec une moyenne totale de 41 %. Les champs osseux avec une lacune d'ostéocytes vides ont été observés et mesurés. La quantité d'os nonvivant variait de 1 à 34 % du volume tissulaire total. La quantité d'os non-vivant diminuait significativement avec le temps de guérison. Ces données suggèrent que la majorité des ostéocytes de l'os monocortical ne survivent pas au greffage. Les résultats indiquent que l'os non-vivant est progressivement remodelé en nouvel os vivant en sept mois après le greffage.

# Zusammenfassung

Das Schicksal von monokortikalen Knochenblöcken, welche in die menschliche Maxilla transplantiert werden: eine histologische und histomorphometrische Studie

Lokale Knochendefekte in der anterioren Maxilla werden normalerweise mit monokortikalen Blöcken aus autologem Knochen aufgebaut, um den Defekt vor der Eingliederung von dentalen Implantaten aufzufüllen. Aufgrund zunehmender Evidenz wird vermutet, dass Osteozyten an der Kontrolle der Knochenremodellierung beteiligt und daher wichtig für die Optimierung der Knochenstrukturen um Implantate und für die Osseointegration der Implantate sind. Es ist jedoch nicht ausreichend bekannt, ob Osteozyten überleben, wenn Knochenblöcke in Defekte transplantiert werden.

Bei 19 Patienten wurden monokortikale Knochenblöcke von der Symphyse in den Defektbereich des Alveolarfortsatzes im Oberkiefer transplantiert. Die Knochentransplantate heilten in einer Zeit zwischen 2.5 und 7 Monaten ein. Während der Implantation wurden mit einer Hohlfräse Knochenbiopsien entnommen und für die Hartgewebshistologie aufgearbeitet. Der Knochen wurde histologisch und histomorphometrisch untersucht, um Einsicht in die Dichte, Vitalität und Remodellierung des Transplantats zu erlangen.

Klinisch waren alle Knochentransplantate erfolgreich eingeheilt. Es konnten keine Implantatmisserfolge gesehen werden und es traten nur geringe Resorptionen auf. Histologisch variierte das Knochenvolumen, ausgedrückt als Prozentsatz Gewebevolumen an der Implantatstelle, von 27% bis 57% mit einem Durchschnitt von 41%. Knochenfelder mit leeren Osteozytenlakunen konnten beobachtet und ausgemessen werden. Die Menge dieses sogenannten nicht-vitalen Knochens variierte zwischen 1% und 34% des totalen Gewebevolumens. Die Menge des nicht-vitalen Knochens nahm signifikant mit der Länge der Einheilzeit ab.

Die Daten lassen vermuten, dass die Mehrzahl der Osteozyten des monokortikalen Knochens die Transplantation nicht überleben. Die Resultate zeigen, dass der nicht-vitale Knochen innert 7 Monaten nach der Transplantation progressiv in neuen vitalen Knochen umgebaut wird.

### Resumen

Los defectos óseos locales en el maxilar anterior se injertan comúnmente con bloques monocorticales de hueso autólogo en orden a restaurar el lugar del defecto antes de la colocación de implantes dentales. Una creciente evidencia sugiere que los osteocitos están involucrados en el control del remodelado óseo y de este modo ser importantes para la optimalización de la estructura ósea alrededor de los implantes y así para la osteointegración de los implantes. Sin embargo, no se conoce bien si los osteocitos sobrevivirán cuando los bloques óseos sean injertados en los defectos.

Hemos injertado a 19 pacientes con bloques de hueso monocortical derivados de la sínfisis al lugar del defecto en el proceso alveolar maxilar. Los injertos óseos se dejaron cicatrizar por un periodo de tiempo que varió entre 2.5 a 7 meses. Durante la implantación se tomaron biopsias óseas usando una fresa de trépano y se procesaron para histología de tejidos duros. Se llevaron a cabo entonces histología ósea e histomorfometría en orden a hacerse una idea acerca de la densidad, viabilidad y remodelado del injerto.

Clínicamente, todos los injertos óseos tuvieron éxito sin fracasos de implantes y se observó poca reabsorción ósea. Histológicamente, el volumen óseo expresado como porcentaje de volumen tisular en el lugar del implante varió del 27% al 57% con una media general del 41%. Se observaron y midieron campos óseos con lagunas óseas vacías. La cantidad de hueso no vital disminuyó significativamente durante el tiempo de cicatrización.

### 要旨:

上顎前歯の局所的骨欠損は、歯牙インプラント 埋入に先立ち欠損再建のため、自家骨のモノコー ティカル骨ブロックを移植することがよく行われ ている。多くのエビデンスによって、骨細胞が骨 のリモデリングの制御に関与しており、インプラ ント周囲の骨構造の最適化及びインプラント骨性 結合にとって重要であることが示唆されている。 しかしながら、骨ブロックを欠損部位に移植した 後に骨細胞が生着するかどうかについてはよく知 られていない。

我々は患者19名において、上顎歯槽突起の欠損 部位に、オトガイ結合部から採取したモノコーテ ィカル骨ブロックを移植した。移植骨の治癒に、 2.5ヶ月から7ヶ月の期間を置いた。インプラ ント埋入時に、骨生検標本をトレファン・バーで 採取し、硬組織の組織学的分析を行った。骨の組 織学評価及び組織形態計測を行い、移植骨の密度 viability 及びリモデリングについて調べた。全て の骨移植は臨床的に成功しており、インプラント の失敗もなく、骨吸収もほとんどおこらなかった。 組織学的には、インプラント部位での%組織量と して表わされる骨量には、27%から57%の幅 があり、平均41%であった。空の骨細胞小腔を ともなう骨領域が観察され、測定された。いわゆ る非生活 (non-vital) 骨の量には、組織総量の1% から34%の幅があった。非生活骨の量は治癒時 間の経過につれて、有意に減少した。 本データは、モノコーティカル骨の骨細胞の大半 は移植後に生着しないことを示唆している。本結 果は、非生活骨は移植後7ヶ月のうちに徐々にリ

モデリングによって新生生活骨に改造されてゆく ことを示している。

### References

- Berggren, A., Weiland, A.J. & Dorfman, H. (1982) The effect of prolonged ischaemia time on osteocyte and osteoblast survival in composite bone grafts revascularized by microvascular anastonoses. *Plastic and Reconstructive Surgery* 69: 290–298.
- Block, M.S. & Kent, J.N. (1997) Sinus augmentation for dental implants: the use of autogenous bone. *Journal of Maxillofacial Surgery* 55: 1281–1286.
- Blomqvist, J.E., Alberius, P., Isaksson, S., Linde, A. & Obrant, K. (1998) Importance of bone graft quality for implant integration after maxillary sinus reconstruction. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 86: 268–274.
- Bronckers, A.L.J.J., Goei, W., Luo, G., Karsenty, G., D' Souza, R.N., Lyaruu, D.M. & Burger, E.H. (1996) DNA fragmentation during bone formation in neonatal rodents assessed by transferase mediated end labelling. *Journal of Bone and Mineral Research* 11: 1281–1291.
- Chugh, S., Marks, D.S., Mangham, D.C. & Thompson, A.G. (1998) Autologous bone grafting in staged scoliosis surgery. The patient as bone bank. *Spine* **23**: 1793–1795.
- Devlin, H., Sloan, P. & Luther, F. (1994) Alveolar bone resorption: a histologic study comparing bone turnover in the edentulous mandible and iliac crest. *Journal of Prosthetic Dentistry* 71: 478–481.
- Ellegaard, B., Karring, T. & Loe, H. (1975) The fate of vital and devitalized bone grafts in the healing of interradicular lesions. *Journal of Periodontal and Restorative Dentistry* 10: 88–97.
- Goldberg, V.M., Shaffer, J.W., Field, G. & Davy, D.T. (1987) Biology of vascularized bone grafts. Orthopedic Clinics of North America 18: 197– 205.
- Goldberg, V.M. & Stevenson, S. (1987) Natural history of autografts and allografts. *Clinical Orthopedics* 225: 7–16.
- Hunt, D.R. & Jovanovic, S.A. (1999) Autologous bone harvesting: a chin graft technique for particulate and monocortical bone blocks. *The International Journal of Periodontics and Restorative Dentistry* 19: 165–174.
- Jensen, J. & Sindet-Pedersen, S. (1991) Autogenous mandibular bone grafts and osseointegrated

implants for reconstruction of the severely atrophied maxilla: a preliminary report. *Journal of Oral and Maxillofacial Surgery* **49**: 1277–1287.

- Kamijou, T., Nakajima, T. & Ozawa, H. (1994) Effects of osteocytes on osteoinduction in the autogenous rib graft in the rat mandible. *Bone* 15: 629–637.
- Kikuyama, A., Fukuda, K., Mori, S., Okada, M., Yamaguchi, H. & Hamanishi, C. (2002) Hydrogen peroxide induces apoptosis of osteocytes: involvement of calcium ion and caspase activity. *Calcified Tissue International* 71: 243–248.
- Kingsmill, V.J., Boyde, A. & Jones, S.J. (1999) The resorption of vital and devitalized bone *in vitro*: significance for bone grafts. *Calcified Tissue International* 64: 252–256.
- Klein-Nulend, J., Van der Plas, A., Semeins, C.M., Ajubi, N.E., Frangos, J.A., Nijweide, P.J. & Burger, E.H. (1995) Sensitivity of osteocytes to biomechanical stress *in vitro*. *FASEB Journal* 9: 441–445.
- Koole, R. (1994) Ectomesenchymal mandibular symphysis bone graft: an improvement in alveolar cleft grafting? *Cleft Palate-Craniofacial Journal* 31: 217–223.
- Listrom, R.D. & Symington, J.S. (1988) Osseointegrated dental implants in conjunction with bone grafts. *International Journal of Oral and Maxillofacial Surgery* 17: 116–118.
- Lundgren, S., Moy, P., Johansson, C. & Nilsson, H. (1996) Augmentation of the maxillary sinus floor with particulate mandible: a histologic and histomorphometric study. *International Journal of Oral & Maxillofacial Implants* **11**: 760–766.
- Misch, C.M. (1997) Comparison of intraoral donor sites for onlay grafting prior to implant placement. *International Journal Oral & Maxillofacial Implants* 12: 767–776.
- Misch, C.M., Misch, C.E., Resnik, R.R. & Ismail, Y.H. (1992) Reconstruction of maxillary alveolar defects with mandibular symphysis grafts for dental implants: a preliminary procedural report. *International Journal of Oral & Maxillofacial Implants* 7: 360–366.
- Montazem, A., Varauri, D.V., St-Hilaire, H. & Buchbinder, D. (2000) The mandibular symphysis

as a donor site in maxillofacial bone grafting: a quantitative anatomic study. *Journal of Oral and Maxillofacial Surgery* **58**: 1368–1371.

- Moran, C.G. & Wood, M.B. (1993) Vascularized bone autografts. Orthopedic Review 22: 187–197.
- Parfitt, M., Drezner, M.K., Glorieux, F.H., Kanis, J.A., Malluchie, H., Meunier, P.J., Ott, S.M. & Recker, R.R. (1987) Bone histomorphometry: standardization of nomenclature, symbols, and units. *Journal of Bone and Mineral Research* 2: 595–610.
- Romeis, B. (1989) *Mikroskopische Technik*, 17th edn, Trichromfärbung nach Goldner, 558. Munchen: Urban & Scharzenberg.
- Smit, T.H. & Burger, E.H. (2002) A case for straininduced fluid-flow as regulation of bone-coupling and osteonal alignment. *Journal of Bone and Mineral Research* 11: 2021–2029.
- Tadjoedin, E.S., De Lange, G.L., Holzmann, P.J., Kuiper, L. & Burger, E.H. (2000) Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. *Clinical Oral Implants Research* 11: 334-344.
- Van de Wijngaert, F.P. & Burger, E.H. (1986) Demonstration of tartrate resistant acid phosphatase in undecalcified glycomethacrylate embedded mouse bone: a possible marker for (pre) osteoclast identification. *Journal of Histochemistry and Cytochemistry* **34**: 1317–1323.
- Verborgt, O., Gibson, G.J. & Shaffler, M.B. (2001) Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue *in* vivo. Journal of Bone and Mineral Research 15: 60–67.
- Widmark, G., Andersson, B. & Ivanoff, C.J. (1997) Mandibular bone graft in the anterior maxilla for single tooth implants. Presentation of a surgical method. *International Journal of Oral and Maxillofacial Surgery* 26: 106–109.
- Zhao, S., Zhang, Y.K., Harris, S., Ahuja, S.S. & Bonewald, L.F. (2002) MLO-Y4 osteocyte-like cells support osteoclast formation and activation. *Journal of Bone and Mineral Research* 17: 2068–2079.