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# Modeling and remodeling of human extraction sockets

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***Abstract***

**Introduction:** The available studies on extraction wound repair in humans are affected by significant limitations and have failed to evaluate tissue alterations occurring in all compartments of the hard tissue defect.

**Aim:** To monitor during a 6-month period the healing of human extraction sockets and include a semi-quantitative analysis of tissues and cell populations involved in various stages of the processes of modeling/remodeling.

**Material and Methods:** Twenty-seven biopsies, representative of the early (2–4 weeks, $n = 10$), intermediate (6–8 weeks, $n = 6$), and late phase (12–24 weeks, $n = 11$) of healing, were collected and analysed.

**Results:** Granulation tissue that was present in comparatively large amounts in the early healing phase of socket healing, was in the interval between the early and intermediate observation phase replaced with provisional matrix and woven bone. The density of vascular structures and macrophages slowly decreased from 2 to 4 weeks over time. The presence of osteoblasts peaked at 6–8 weeks and remained almost stable thereafter; a small number of osteoclasts were present in a few specimens at each observation interval.

**Conclusions:** The present findings demonstrated that great variability exists in man with respect to hard tissue formation within extraction sockets. Thus, whereas a provisional connective tissue consistently forms within the first weeks of healing, the interval during which mineralized bone is laid down is much less predictable.

Key words: alveolar ridge; bone healing; histomorphometric analysis; tissue modeling/remodeling; tooth extraction

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The alveolar process forms during tooth eruption (Schroeder 1986) and undergoes atrophy after the loss of single or multiple teeth (e.g. Pietrokovski & Massler 1967, Johnson 1969, Schropp et al. 2003, Pietrokovski et al. 2007). As a rule, the resorption of the buccal compartment of the ridge – after tooth removal – is more pronounced than the amount of tissue that is lost in the lingual/palatal portion. The alteration of the ridge occurs concomitantly with the healing of the soft and hard tissue wound but the process of remodeling may continue also after the termination of *de novo* bone formation in the socket(s) (Schropp et al. 2003).

Processes involved in the healing of an extraction socket have been studied in different animal models (e.g. Kuboki et al. 1988, Lin et al. 1994, Lekic et al. 2001, Cardaropoli et al. 2003, 2005, Kanayama et al. 2003, Sato & Takeda 2007). In a recent study using a dog model, Cardaropoli et al. (2003) explored the healing of the coronal, central and apical compartments of fresh extraction sockets during a 6-month interval. The authors observed that in this model, the socket already after 4 weeks of healing was filled with woven bone and that after 2 months this immature bone had been replaced with lamellar bone and marrow. Concomitant with these intra-alveolar healing events, the buccal wall of the socket underwent marked resorption. Thus, in comparison to the lingual wall of the socket, the height of its buccal counterpart was reduced on the average 2.5 mm (Araujo & Lindhe 2005).

Tissue formation after tooth extraction was also studied in human models (Claflin 1936, Mangos 1941, Christopher 1942, Amler et al. 1960, 1964, 1969, Boyne 1966, Evian et al. 1982). Amler (1969) examined new tissue formation in the marginal portion of extraction sites from human volunteers at healing

**Conflict of interest and source of funding statement**

The authors declare that they have no conflict of interest.

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intervals extending from 2 to 32 days. He concluded that the blood clot that initially filled the entrance of the socket was first replaced with granulation tissue. After 1 week of tissue modeling, osteoid formation had begun and after about 6 weeks, the marginal portion of the socket harboured islands of immature woven bone. However, the human studies cited above (i) included tissue samples from systemically diseased individuals or even cadavers (Claflin 1936, Mangos 1941), (ii) had an unclear experimental protocol (Christopher 1942), (iii) evaluated only few tissue samples and/or (iv) short observation intervals (Amler et al. 1960, Boyne 1966, Evian et al. 1982).

On the basis of the above concerns, a study was designed to (i) monitor the healing of human extraction sockets and (ii) include a semi-quantitative analysis of tissues and cell populations involved in various stages of the healing process.

## Material and Methods

### Patient and site selection

Subjects were considered eligible for the study if they were $\geqslant$18-years old and if they did not suffer from systemic disease. Pregnant or lactating women, and subjects taking (past or currently) drugs influencing bone metabolism were excluded. The study was approved by the Ethical Committee of the University of Ferrara. All included subjects provided an informed consent before participation.

The following tooth sites were considered: single rooted-teeth, molars or premolars with fused roots or single roots of molars. Biopsy was consistently performed in conjunction with periodontal surgery or implant placement in the extraction site. Teeth with (i) periapical lesion were excluded as well as (ii) sites with limited depth ($<$5 mm) of the remaining sockets after tooth removal.

### Extraction and post-extraction protocol

Full thickness buccal and lingual mucoperiosteal flaps were elevated. The tooth was carefully removed with the use of elevators. No ostectomy or osteoplasty was performed. No graft or membrane device was used to augment the site. Flaps were sutured with 5/0 silk or Vycril® suture (ETHICON, Johnson & Johnson, Cincinnati, OH, USA). The wound was left to heal for primary intention. When needed, partial thickness dissection in the most apical portion of the flap was performed in order to coronally advance the flap. Local antimicrobial therapy (chlorhexidine 0.12% mouthrinse; 10 ml for 60 s t.i.d. for 7 days) was prescribed. Sutures were removed 1 week postoperatively. If a local complication (e.g. rupture of soft tissue wound) occurred during healing and/or systemic antibiotic therapy had to be prescribed, the site was excluded from the study.

### Localization of the extraction site

Before tooth extraction, an alginate impression (Xantalgin®; Heraeus Kulzer S.r.l., Milan, Italy) was obtained of the experimental area. On a cast model, a resin/silicon stent was prepared and adapted on the dentition adjacent to the tooth to be removed. A canal parallel with the long axis of the tooth was prepared in the stent. This allowed the operator to properly identify the socket site during biopsy.

Immediately after tooth extraction, the distance from the stent to the bottom of the socket was measured. This was made with a UNC-15 periodontal probe. This recording was used in order to ensure that, at the time of biopsy, the trephine bur would collect the newly formed tissue only.

### Collection and storage of the specimens

The timing of the biopsy procedure was selected in accordance with the clinical need for the surgical procedure (i.e. periodontal surgery or implant positioning) involving the extraction socket. At the time of biopsy retrieval, local infiltration of anaesthetic was administered. Whenever the concomitant surgical procedure required a full-thickness flap, a crestal incision was performed and a mucoperiosteal flap was elevated on both buccal and lingual/palatal side with a microsurgical periosteal elevator (P-TROM; Hu-Friedy, Chicago, IL, USA). When this was not the case (e.g. implant positioning with a flapless approach), the soft tissues overlying the extraction socket were included in the biopsy. The tissue specimens were collected with a trephine bur (internal diameter 2 mm; Hu-Friedy) from the healing sockets. The depth of the trephine bur insertion was related to the measurements previously made by the stent (see ''Localization of the extraction site''). The tissue was obtained from the centre of the socket, with the insertion axis of the trephine kept parallel to the long axis of the adjacent tooth. The apical portion of the specimen was marked with a fine indelible chine pen.

### Histological and immunohistochemical processing

The specimens were immediately placed in a 10% formaldehyde fixative, decalcified in ethylene diaminetetraacetic acid dehydrated in increasing concentrations of ethanol, embedded in paraffin and cut in the sagittal plane with the microtome set at 5 $\mu$m. From each biopsy, between 10 and 20 sections representing the central part of the socket were selected for histological and immune-histochemical examinations.

One section was stained in haematoxyline–eosine, and examined in a microscope (Leitzs DM-RBE Microscope; Leica, Wetzlar, Germany) equipped with an image system (Q-500 MCs; Leica).

The total cross-section area of the tissue sample was outlined and the size determined by planimetry. Subsequently, the areas (proportions of the cross-section area) occupied by (1) clot (erythrocytes, leukocytes embedded in a fibrin network), (2) granulation tissue (rich in newly formed vascular structures, abundance of inflammatory cells such as neutrophils, macrophages, lymphocytes as well as erythrocytes), (3) provisional matrix (densely packed mesenchymal cells, collagen fibres and vessels but no or only scattered inflammatory cells), (4) woven bone (finger-like projections of immature bone embedded in a primary spongiosa) and (5) lamellar bone and marrow, were calculated.

A series of paraffin sections were deparaffinized, treated with TE-buffer (pH 9) or Proteinase K (Dakacytomation, Glostrup, Denmark) and washed in Tris-buffered saline (TBS) for 3 min. Endogenous peroxidase activity was blocked with a peroxidase solution for 10 min. and subsequently rinsed for 3 min. with TBS. A panel of monoclonal antibodies diluted in TBS was applied for 30 min. and washed in TBS for 2 $\times$ 3 min. The sections were incubated with a peroxidase labelled polymer for 30 min. and washed in TBS for 3 $\times$ 3 min. Diaminobenzidine tetrahydrochloride (DAB) was used as

*Table 1*. Isotypes and dilutions of the monoclonal antibodies used for immuno-histochemical stainings

| Antibodies (clone) | Specificity | Dilutions | Isotype | Source |
|---|---|---|---|---|
| CD 31 (JC70A) | Endothelial cell | 1:20 | IgG1 | Dakocytomation, Glostrup Denmark |
| BMP7 (164313) | BMP7 | 1:20 | IgG1 | R&D Systems, Europe Ltd Oxon, UK |
| Osteocalcin (190125) | Osteoblast | 1:10 | IgG1 | R&D Systems, Europe Ltd |
| CD68 | Macrophage | 1:20 | IgG1 | Diagnostic Biosystems, Pleasanton, CA, USA |
| RANK/TNFRSF11A (80707) | Osteoclast | 1:20 | IgG2A | R&D Systems, Europe Ltd |

substrate/chromogen (EnVision+® System-HRP (DAP) Kit; Dakocytomation). The sections were rinsed in TBS and distilled water, counterstained in Mayer's haematoxyline stain and mounted. For each antibody tested, one section was incubated in serum and served as a negative control.

The various antibodies were used to identify vascular (endothelial cells) structures (CD31), osteoblasts (BMP-7, Osteocalcin) osteoclasts (RANK/TNFRSF 11A) and macrophages, giant cells (CD68). The monoclonal antibodies used, their isotypes and dilutions are presented in Table 1.

#### Semi-quantitation

The morphometric assessment on CD31-stained sections was conducted according to methods described earlier (Schroeder & Münzel-Pedrazzoli 1973). Briefly, the cross-section area of the tissue samples was determined ($\times 1.6$) and vascular structures were subsequently identified and enumerated ($\times 10$). The numerical density of vascular units per area unit ($mm^2$) of available tissue was then calculated.

A semi-quantitative analysis of BMP-7-, osteocalcin- and CD68-positive cells was conducted. For each investigated protein/cell type, a score ranging from 0 (absent) to 4 (large amounts) was determined.

*BMP-7 and osteocalcin*

BMP-7 and osteocalcin scores were related to the percentage of the periphery of the woven bone projections that showed the presence of BMP-7- and osteocalcin-stained cells, respectively.

| | |
|---|---|
| 1 | $<10\%$ |
| 2 | $>25\%$ but $<40\%$ |
| 3 | $>50\%$ but $<75\%$ |
| 4 | $>80\%$ |

*CD68*

CD68 score was related to the number of CD68-stained cells in the entire tissue sample.

| | |
|---|---|
| 1 | $<20$ cells |
| 2 | 30–50 cells |
| 3 | 50–100 cells |
| 4 | $>200$ cells |

All the histologic evaluations and histomorphometric measurements were carried out by a single trained and calibrated examiner (B. L.). Data were expressed as mean ± SD.

## Results

Twenty-four patients (10 males and 14 females, range 34–72 years) were included. Twenty-two patients contributed with one, one patient with two and one patient with three extraction sites. All the biopsy procedures were performed without any major intra-operative complications. Occasionally, the sampled tissue fragmented into two to three separate pieces during biopsy harvesting, especially when newly formed hard tissue was present in the extraction socket. When this was the case, all the pieces were retrieved, oriented, stored together and analysed as a single sample.

The location of the extraction sites was as follows: 1 lateral incisor, 1 canine, 19 premolars, 6 molars. Twelve teeth were obtained from the upper jaw and fifteen from the lower jaw. Teeth were extracted due to caries ($n = 8$), vertical root fracture ($n = 9$), failure of endodontic therapy ($n = 3$), periodontal disease ($n = 5$), or for prosthetic reasons ($n = 2$).

Of the 27 biopsies that were available for analysis, 10 were sampled to represent early healing (2–4 weeks), six intermediate (6–8 weeks), and 11 a later phase (12–24 weeks) of healing.

#### Gross morphological characteristics of the tissue samples

In most specimens examined, it was possible to distinguish between different types of tissues. Thus, in some regions the biopsy could disclose features typical of granulation tissue whereas in other regions of the same specimen, tissue modeling was more advanced and areas including provisional matrix and/or woven bone could be identified.

*Granulation tissue* was characterized by the presence of large numbers of vascular structures in a connective tissue comprising mesenchymal cells and infiltrates of leukocytes (neutrophilic leukocytes, macrophages and lymphocytes) (Fig. 1). The inflammatory cells were mainly present in peri-vascular compartments (Fig. 2) but occurred also in areas more distant to vascular structures.

*Provisional matrix* contained densely packed mesenchymal cells present in a collagen-rich connective tissue matrix (Fig. 3). Vascular structures (Fig. 4) were abundant but only few infiltrates of leukocytes (macrophages and lymphocytes) could be observed. Osteoblasts (osteocalcin-stained cells) occurred in peri-vascular locations.

*Woven bone* occurred as fingerlike projections of mineralized tissue (Fig. 5) in a connective tissue matrix. The ridges of woven bone were lined with osteoblasts and contained large numbers of osteocytes (Fig. 6). The trabeculae of mineralized bone occurred in the vicinity of, often surrounding, one or several vascular structures (Fig. 7). Osteoclasts (RANK/TNFRSF 11A-positive cells) occurred in discrete areas at the surface of woven bone trabeculae and were consistently present in Howship's lacunae (Fig. 8).

Only one section (12 weeks of healing) (Fig. 9) comprised *lamellar bone and marrow*. The mineralized bone harboured secondary osteons whereas the bone marrow was rich in vessels, adipocytes, mesenchymal cells and inflammatory cells (macrophages, lymphocytes). Osteoclasts were present in several areas at the surface of the mineralized bone.

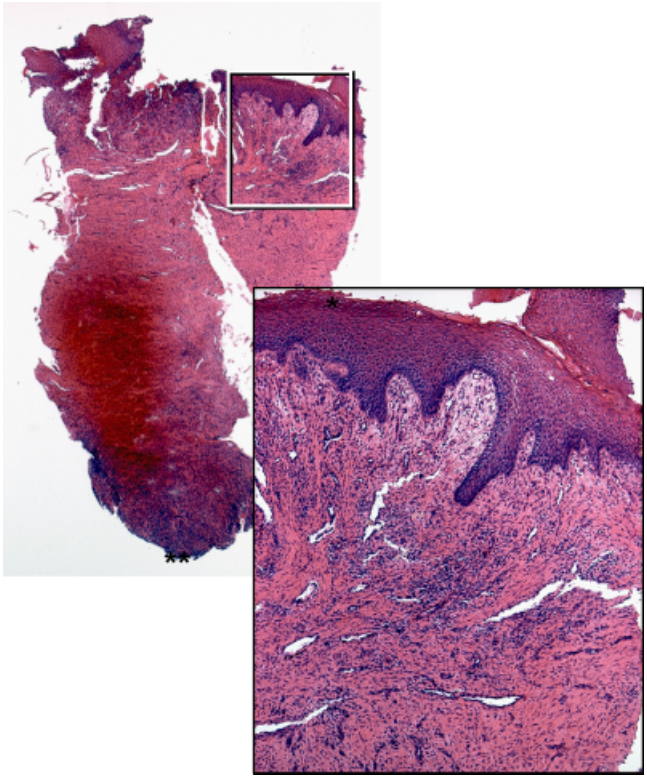

*Fig. 1*. A biopsy obtained after 3 weeks of healing. The tissue is rich in vessels, fibroblasts and inflammatory cells and is characterized as *granulation tissue*. Original magnification ×2.5. Inset: large amounts of inflammatory cells can be observed immediately beneath the oral epithelium. Original magnification ×10. H & E stain.

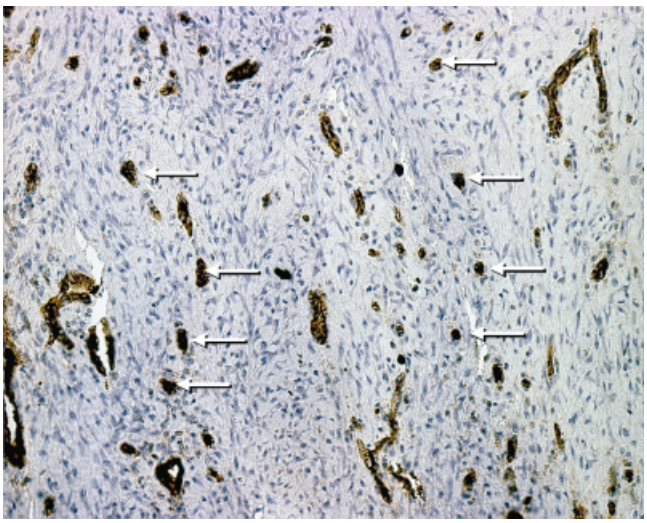

*Fig. 2*. Detail from Fig. 1 illustrating vascular structures that have been stained with CD 31. Note the large amount of vessels of varying diameters including several vascular sprouts (arrows). Original magnification ×10.

#### Histomorphometric measurements

The composition of each tissue sample is shown in Table 2. Only in samples representing the early interval of healing (2–4 weeks) could few, scattered erythrocytes be observed in various parts of the sections. However, no definitive clot formation could be distinguished.

#### Granulation tissue

In 4 out of 10 samples representing the early healing interval, granulation tissue could be observed (Table 2). In these specimens, the richly vascularized, heavy infiltrated tissue occupied between 67% and 100% of the samples examined. Six of the sections contained no granulation tissue. The overall mean area occupied by granulation tissue in this healing interval was 35.9 ± 47.2% (Fig. 10).

Only one of the six biopsies representing the intermediate healing interval (6–8 weeks; Table 2) contained a typical granulation tissue, whereas no specimen in the 12–24 week interval harboured this type of immature tissue.

#### *Provisional matrix*

A tissue rich in mesenchymal cells, collagen fibres and vascular structures was found in 7 out of 10 specimens from the early healing interval. In these specimens, the provisional matrix occupied between 33% and 100% of the tissue examined with mean value 57.2 ± 44.2% (Table 2). All sections from the intermediate healing period harboured differently large zones (between 27% and 98%; mean value 62.2 ± 23.8%) of provisional matrix. Ten out of eleven biopsies from the late healing interval contained a typical provisional matrix tissue. In these sections, between 35% and 92% of the area examined contained this type of tissue with mean value 58.5 ± 24.5%.

#### *Woven bone and lamellar bone*

Areas occupied by woven bone could be observed already in specimens from the 2–4 week interval. Thus, in 6 out of 10 biopsies (Table 2) fingerlike projections of newly formed bone could be identified. When present in the sections, this immature bone occupied between 2% and 34% of the tissue area (mean 6.9 ± 10.5%; Fig. 10).

In practically all specimens representing the intermediate and late healing interval, woven bone was present and occupied between 2% and 73% of the tissue examined (mean value 6–8 weeks: 34.0 ± 24.6%; 12–24 weeks: 32.4 ± 18.4%). Lamellar bone and marrow was found in only one biopsy and this tissue represented the 12–24 week healing interval (Table 2).

#### Immunohistochemical analysis

Table 3 shows the presence of positive cells for each staining.

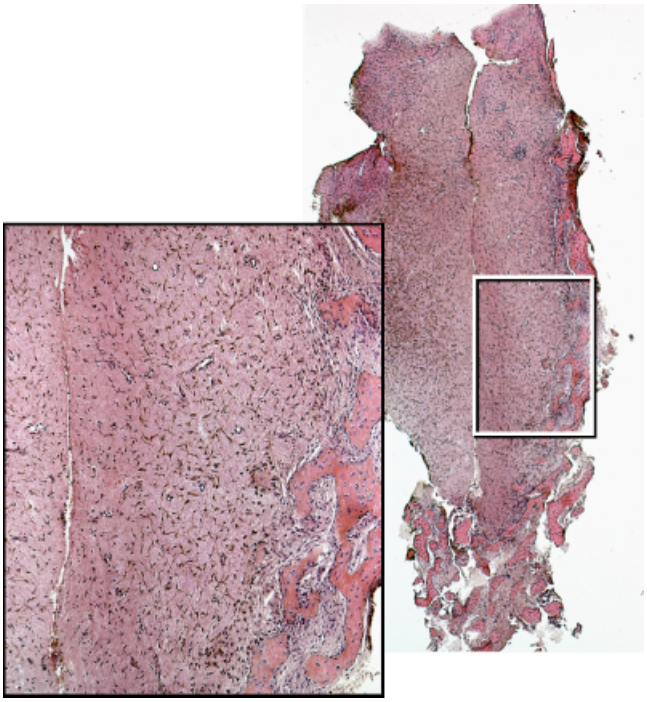

*Fig. 3*. A biopsy obtained after 4 weeks of healing. The *provisional matrix* comprises mesenchymal cells densely packed fibres and vessels. Only few inflammatory cells can be observed. Original magnification ×2.5. Inset: at the lateral border of the section, minute areas of woven bone formation can be identified. Original magnification ×10. H & E stain.

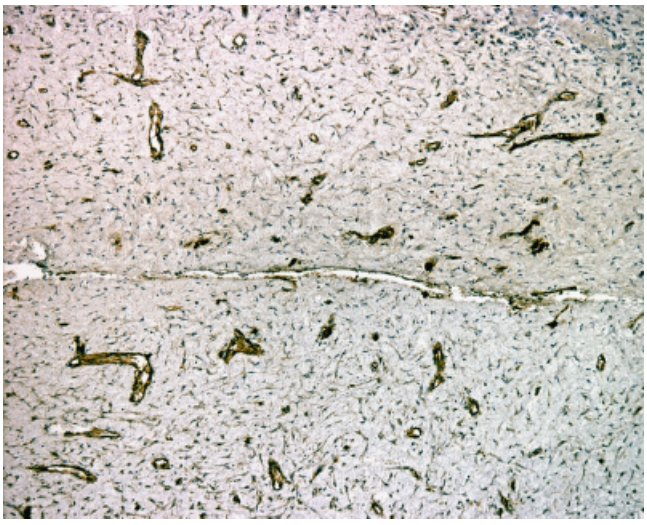

*Fig. 4*. Detail from Fig. 3 illustrating vascular structures that have been stained with CD 31. Original magnification ×10.

The number of CD31 positive cells per area unit ($mm^2$) was 33.0 ± 12.2 at 2–4 weeks, 27.2 ± 5.6 at 6–8 weeks and 19.0 ± 8.8 at 12–24 weeks.

BMP-7 score shifted from 1.5 ± 0.6 at 2–4 weeks to 2.8 ± 1.0 at 6–8 weeks, and 2.1 ± 1.2 at 12–24 weeks.

Osteocalcin score shifted from 1.4 ± 0.6 at 2–4 weeks to 2.5 ± 1.0 at 6–8 weeks, and 1.9 ± 0.8 at 12–24 weeks.

CD68 score shifted from 2.8 ± 1.0 at 2–4 weeks to 2.1 ± 1.1 at 6–8 weeks, and 1.1 ± 0.4 at 12–24 weeks.

RANK/TNFRSF 11A-positive cells were detected in low numbers (one to two cells) in only 3 out of 10 specimens at 2–4 weeks. At 6–8 weeks, only few (one to three cells) osteoclasts were found in five out of the six samples. In one sample, however, a large number of osteoclasts (>10 cells) were stained. In specimens representing 12–24 weeks, few osteoclasts were found in 6 out of 11 samples; i.e. five were negative.

## Discussion

The overall findings from the present study demonstrated that the healing of an extraction socket, as disclosed in our panel of subjects, followed a pattern similar to that previously described from histological examinations of tissues harvested from socket sites in man and animals (for review see Chen et al. 2004, Cardaropoli 2006). The process of bone modeling/remodeling described in the present biopsy samples has several features in common with intra-membraneous bone formation (Whistson 1994) and bone formation that occurs in membrane protected defects in the alveolar ridge (Schenk et al. 1994). Data also disclosed that (i) the rate of healing varied markedly between subjects; (ii) the process of remodeling, i.e. the replacement of woven bone with lamellar bone and marrow was slow. Only 1 of the 11 specimens representing 12–24 weeks of healing comprised mature bone, i.e. lamellar bone and marrow; (iii) density of vascular structures was high at 2–4 and 6–8 weeks and tended to decrease in the 12–24 week specimens; (iv) presence of osteoblasts peaked at 6–8 weeks and remained almost stable thereafter; (v) macrophages slowly decreased from 2 to 4 weeks over time and (vi) osteoclasts were present in only few specimens at each observation interval.

Immediately after tooth extraction, the socket fills with blood and clot formation occurs (Amler 1969, Cardaropoli et al. 2003). In the present material, erythrocytes scattered in between mesenchymal cells were frequently observed in the tissue from biopsies representing 2–4 weeks, although typical clot formations (erythrocytes, platelets and leukocytes entrapped in a dense fibrin network) could not be observed in any of the tissues examined. This finding is in agreement with data presented by Amler et al. (1960) and Amler (1969) who documented that the blood clot that first filled the socket space was almost entirely remodeled within the first week after tooth removal. Furthermore,

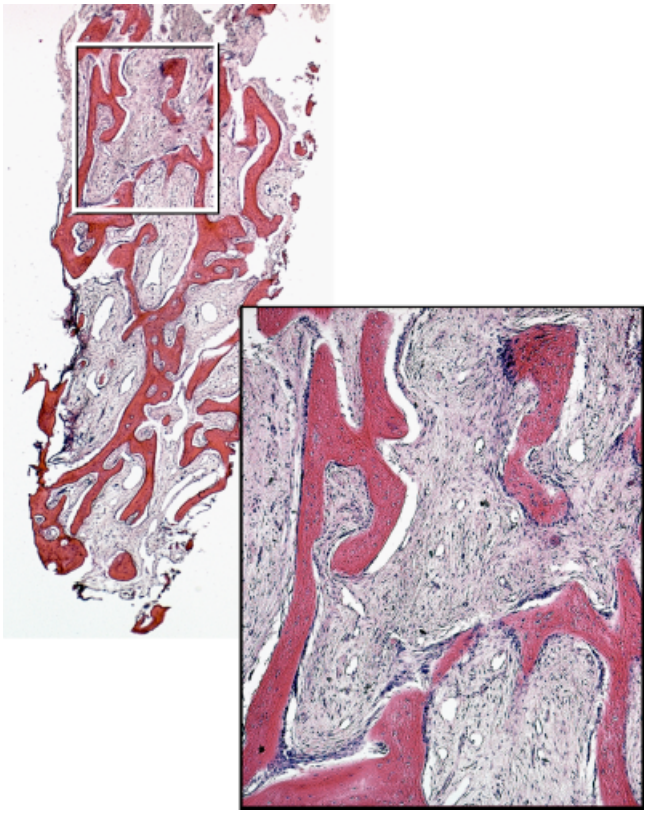

*Fig. 5*. Decalcified section obtained from a biopsy sampled after 6 weeks of healing. Note the presence of trabeculae of immature *woven bone* that occur in a cell and fibre-rich provisional matrix. Original magnification ×2.5. Inset: the projections of woven bone are enclosing vascular structures to form primary osteons. Original magnification ×10. H & E stain.

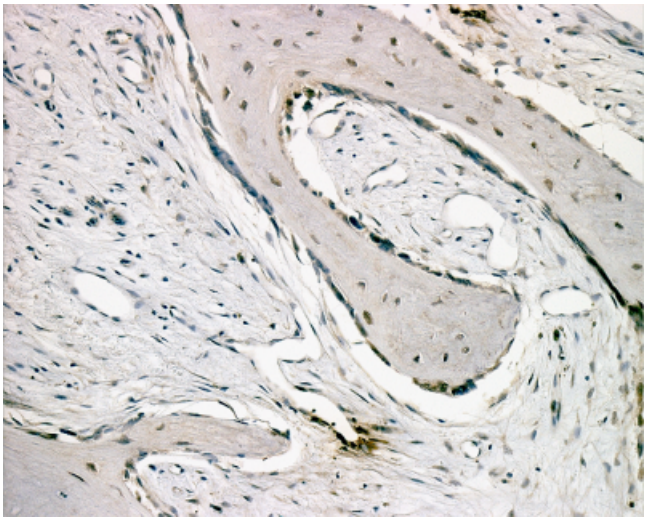

*Fig. 6*. Detail from Fig. 5 illustrating osteoblasts that line the newly formed woven bone as well as osteocytes within the immature bone. Osteocalcin staining. Original magnification ×20.

most of the granulation tissue that occurred in samples representing the early phase (2–4 weeks) of healing, had in the interval between the early and intermediate healing phase (6–8 weeks) apparently been replaced with provisional matrix and woven bone. This observation corroborates findings by Amler et al. (1960) and Evian et al. (1982) who reported that between the first and third week of socket healing there was a progressive replacement of granulation tissue with connective tissue. Provisional matrix and woven bone also dominated in biopsies from the late phase of healing (12–24 weeks) of the current study, and only one specimen (representing 12 weeks) included substantial amounts of lamellar bone and marrow. In other words, whereas tissue modeling in the human extraction sites appeared to be a comparatively fast process, remodeling of the newly formed hard tissue was seemingly slow.

The observation that bone remodeling of extraction sites in man is a slow process is in many respects consistent with data presented by Schropp et al. (2003). They used clinical and radiographic means to monitor tissue alterations within and outside extraction sites in the premolar and molar regions of 46 patients. The data described by the authors disclosed that the dimensions of the extraction sites were markedly reduced after tooth removal. Thus, at 3 months >30% and at 12 months >50% of the buccal–lingual/palatal width of the marginal portion of the ridge was lost after tooth removal. Moreover, it was noted that large amounts of mineralized tissue formed in the sockets during the first 6 months whereas in the interval between 6 and 12 months, the amount of newly formed mineralized tissue was substantially reduced (woven bone was replaced with lamellar bone and marrow). The findings by Schropp et al. (2003) thus demonstrate that healing of an extraction site in man proceeds with the resorption of the marginal socket walls as well as with varying degree of hard tissue fill within the socket. It is important to emphasize that the biopsy technique used in the current study limited the analysis to the intra-alveolar events.

In a recent publication, Chen et al. (2004) reported on osseous regeneration in human extraction sockets and included four studies with histologic documentation in their review (Amler et al. 1960, 1969, Boyne 1966, Evian et al. 1982). Amler (1969) claimed that after about 40 days of healing two-third of the sockets were filled with mineralized bone, whereas Evian et al. (1982) stated that the sockets were completely filled with bone after 10 weeks of healing. The findings from the current study could only in part corroborate the above findings. Thus, in specimens obtained between 6 and 8 weeks woven bone

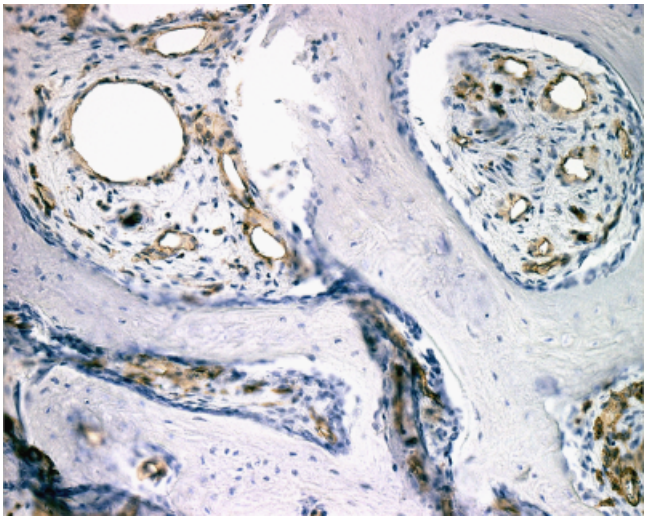

*Fig. 7*. Detail from Fig. 5. The tissue was stained with CD 31 and illustrates vascular structures that reside in the centre of primary osteons. Original magnification × 20.

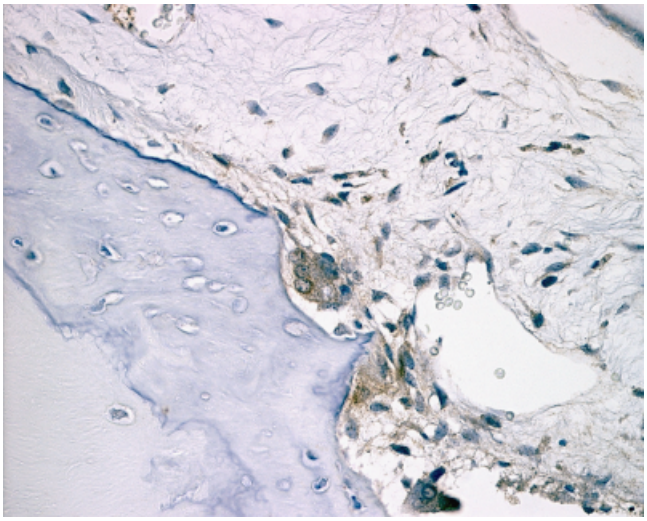

*Fig. 8*. Detail from Fig 5. Note the presence of osteoclasts on the surface of newly formed bone. Stain: Rank/TNFRSF 11A. Original magnification × 40.

occupied about 35% of the tissue sample, whereas after 12 and 24 weeks of wound healing only about 41% of the biopsy harvested from the socket comprised mineralized bone.

Various aspects of healing of extraction sockets were studied in the dog model (for review see Chen et al. 2004, Cardaropoli 2006). In such carefully monitored animal experiments, (i) the teeth were carefully removed from periodontally non-compromised sites and (ii) healing was analysed in biopsies containing not only the hard tissue defect but in addition the surrounding bone walls (mesial, distal, buccal, lingual). In such experiments, new tissue formation in various parts of the socket followed a well-defined course. Thus, the clot that formed immediately after tooth removal was in the first few days consistently replaced with granulation tissue that already after 1 week in part had been replaced with a cell rich provisional matrix. After 2 weeks of healing, large portions of the apical and lateral compartments of the socket were occupied by woven bone and after 4 weeks, the process of modeling was advanced. Hence, in the interval between 4 and 8 weeks, the newly formed immature woven bone was replaced with lamellar bone and marrow. In the tissue samples harvested from the human extraction sites included in the current study, this rather distinct pattern of tissue modeling/remodeling was difficult to identify. In contrast, the results from the histological examinations revealed great variation between samples with respect to tissue formation and maturation. The reason for this variation is presently not understood but may be linked to subject-related factors, pre-existing damage to the tooth and its supporting tissues (reason for tooth extraction), trauma inflicted to the tissues in conjunction with tooth removal, the size of the hard tissue defect, etc.

In our study, immunohistochemical stainings were used to investigate the time-dependent changes in density of the main cell types involved in post-extraction wound healing. The density of endothelial cells, assumed as corresponding to the number of CD31-positive cells, was decreased at 12–24 weeks with respect to 2–4 weeks and 6–8 week observations. This finding can be explained by the progressive remodeling of the highly vascularized granulation tissue and its subsequent replacement by provisional matrix.

The histomorphometric analysis included the use of antibodies against BMP-7 and osteocalcin. For these measurements, only positive cells lining the newly formed trabeculae of woven bone were considered in order to limit the study to the cells directly involved in the bone forming and remodeling process. For both antibodies, the density of positive cells increased from 2–4 weeks to 6–8 weeks and tended to decrease at 12–24 weeks. The increase in BMP-7 positive cells between the early and intermediate healing phase can be attributed to an increased bone modeling and remodeling activity, leading to the deposition of woven bone from provisional matrix. In this respect, previous studies demonstrated that BMP-7 is highly expressed during the early stages of bone repair after a fracture (Spector et al. 2001, Kloen et al. 2003). This protein continues to be expressed during the process of bone remodeling, albeit less prominently, and the return of BMP-7 density to baseline value coincides with the histological appearance of mature lamellar bone (Spector et al. 2001). In this respect, the limited presence of lamellar bone in the 12–24 week specimens observed in the current study was associated with a tendency for decrease in BMP-7 levels.

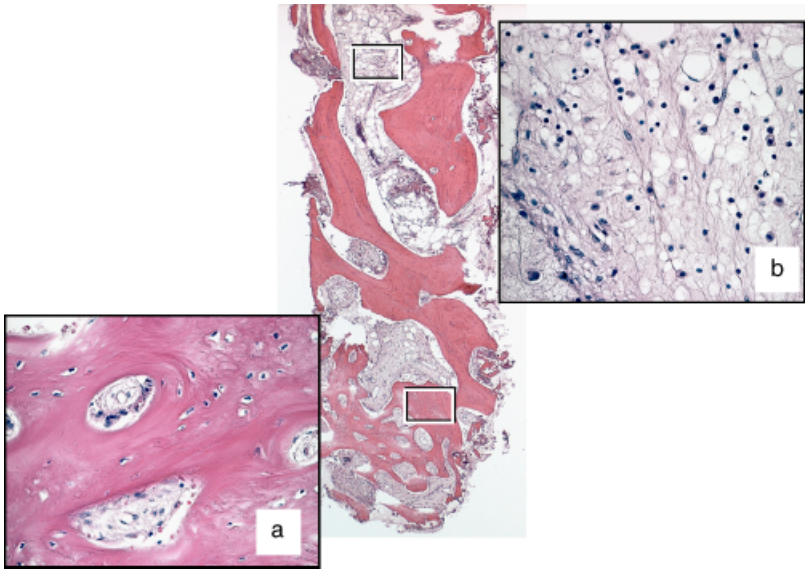


*Fig. 9.* Biopsy obtained from an extraction wound representing 12 weeks of healing. The tissue comprises more mature bone; woven bone and lamellar bone that reside in a non-mineralized matrix. Original magnification ×2.5. H & E stain. Inset A: note the presence of secondary osteons in lamellar bone. Original magnification ×40. Inset B: large number of inflammatory cells among adipocytes in the newly regenerated bone marrow. Original magnification ×40.

*Table 2.* Composition (percentage) of each sample with respect to clot, granulation tissue, provisional connective tissue, woven bone and lamellar bone/bone marrow

| Sample no. | Clot (%) | Granulation tissue (%) | Provisional connective tissue (%) | Woven bone (%) | Lamellar bone/bone marrow (%) |
|---|---|---|---|---|---|
| 2–4 weeks | | | | | |
| #1 | 0 | 100 | 0 | 0 | 0 |
| #2 | 0 | 92 | 0 | 8 | 0 |
| #3 | 0 | 0 | 89 | 11 | 0 |
| #4 | 0 | 0 | 89 | 11 | 0 |
| #5 | 0 | 0 | 97 | 3 | 0 |
| #6 | 0 | 0 | 100 | 0 | 0 |
| #7 | 0 | 0 | 66 | 34 | 0 |
| #8 | 0 | 100 | 0 | 0 | 0 |
| #9 | 0 | 67 | 33 | 0 | 0 |
| #10 | 0 | 0 | 98 | 2 | 0 |
| 6–8 weeks | | | | | |
| #1 | 0 | 0 | 27 | 73 | 0 |
| #2 | 0 | 23 | 57 | 20 | 0 |
| #3 | 0 | 0 | 53 | 47 | 0 |
| #4 | 0 | 0 | 76 | 24 | 0 |
| #5 | 0 | 0 | 62 | 38 | 0 |
| #6 | 0 | 0 | 98 | 2 | 0 |
| 12–24 weeks | | | | | |
| #1 | 0 | 0 | 52 | 48 | 0 |
| #2 | 0 | 0 | 0 | 0 | 100 |
| #3 | 0 | 0 | 92 | 8 | 0 |
| #4 | 0 | 0 | 84 | 16 | 0 |
| #5 | 0 | 0 | 67 | 33 | 0 |
| #6 | 0 | 0 | 35 | 65 | 0 |
| #7 | 0 | 0 | 64 | 36 | 0 |
| #8 | 0 | 0 | 58 | 42 | 0 |
| #9 | 0 | 0 | 60 | 40 | 0 |
| #10 | 0 | 0 | 64 | 36 | 0 |
| #11 | 0 | 0 | 68 | 32 | 0 |

The osteocalcin expression paralleled BMP-7 expression during the entire observation interval. Osteocalcin is an osteoblast-specific marker, being spatially confined to osteoblasts actively laying down new osteoid or remodeling bone (Paccione et al. 2001). Because of its progressive accumulation in the maturing bone matrix, osteocalcin represents a late marker of bone formation. In fact, a greater expression of osteocalcin-positive cells can be observed in the mature bone matrix than in newly formed bone (Ivanovski et al. 2000, Reinhardt et al. 2005). It was demonstrated that osteocalcin, used as a marker to define alveolar bone turn-over in humans, was negatively correlated with increasing radiographic density of experimentally produced bone cores, i.e. the higher the osteocalcin expression the less dense was the amount of new mineralized bone (Reinhardt et al. 2004).

For obvious reasons, the current study had a cross-sectional design. Most patients contributed with only one biopsy from one healing interval. Thus, it can only be assumed that an immature tissue identified in biopsies from 2 to 4 weeks interval would have matured to form bone if continued healing would have been allowed. Furthermore, cells identified as osteoblasts or pre-osteoblasts – in sections stained with monoclonal antibodies for osteocalcin and BMP-7 – varied in numbers from one biopsy to the next (Table 3) but reached peak values in sections representing 6–8 weeks of healing. In such sections, however, a large number of typical mesenchymal cells were negative for the monoclonal antibodies used. This may indicate that such cells were not in an active bone-forming phase or represented populations of cells not involved in the formation of bone. It is a well-known fact that cells that repopulate a wound determine the nature of the tissues formed (Melcher 1976). Thus, it cannot be taken for granted that tissue portions in the present biopsy sample were occupied by provisional matrix would become replaced with woven bone.

In our study, osteoclasts, when detected, were present in low numbers (one to three cells) at each observation interval. In a dog study by Cardaropoli et al. (2003), a substantial remodeling activity with an increased number of osteoclasts was observed at 1 month after tooth extraction, leading to a decrease in the area occupied by mineralized bone. In humans, a similar remodeling activity was observed not before 6 months after tooth removal, i.e. at a longer observation interval with respect to our study (Schropp et al. 2003). Therefore, it may be hypothesized that our study, due to earlier observation

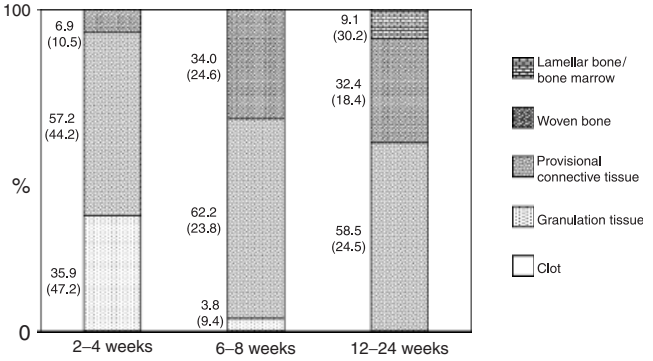


*Fig. 10.* Distribution (mean %) of the tissue components (clot, granulation tissue, provisional connective tissue, woven bone, lamellar bone/bone marrow), calculated on all samples for each observation interval. The standard deviation is shown in parenthesis.

*Table 3.* Density of positive cells for immunohistochemical stainings (CD31, BMP-7, osteocalcin, CD68)

| Sample no. | CD31 (no. of cells/mm$^2$) | BMP-7 (score) | Osteocalcin (score) | CD68 (score) |
|---|---|---|---|---|
| 2–4 weeks | | | | |
| #1 | 38.6 | 0.5 | 1.5 | 4.0 |
| #2 | 27.8 | 1.5 | 1.0 | 3.5 |
| #3 | 25.6 | 2.0 | 2.0 | 1.0 |
| #4 | 23.5 | 2.0 | 2.0 | 1.5 |
| #5 | 30.4 | 2.0 | 2.0 | 3.0 |
| #6 | 35.8 | 0.5 | 1.0 | 3.5 |
| #7 | 16.7 | 1.5 | 1.0 | 2.0 |
| #8 | 61.5 | 1.0 | 2.0 | 3.0 |
| #9 | 31.3 | 1.5 | 0.5 | 3.5 |
| #10 | 38.7 | 2.0 | 0.5 | 3.0 |
| Mean | 33.0 | 1.5 | 1.4 | 2.8 |
| SD | 12.2 | 0.6 | 0.6 | 1.0 |
| 6–8 weeks | | | | |
| #1 | 36.1 | 3.0 | 3.0 | 1.5 |
| #2 | 24.0 | 3.0 | 2.0 | 2.0 |
| #3 | 20.9 | 3.0 | 3.0 | 1.0 |
| #4 | 23.0 | 4.0 | 4.0 | 2.0 |
| #5 | 29.7 | 3.0 | 2.0 | Not available |
| #6 | 29.4 | 1.0 | 1.0 | 4.0 |
| Mean | 27.2 | 2.8 | 2.5 | 2.1 |
| SD | 5.6 | 1.0 | 1.0 | 1.1 |
| 12–24 weeks | | | | |
| #1 | 27.0 | 4.0 | 3.0 | Not available |
| #2 | 13.7 | 4.0 | 3.0 | 0.5 |
| #3 | 38.7 | 1.0 | 1.0 | 0.5 |
| #4 | 12.7 | 1.0 | 1.0 | 1.0 |
| #5 | 23.3 | 3.0 | 2.0 | 1.5 |
| #6 | 10.3 | 0.5 | 1.0 | Not available |
| #7 | 14.8 | 1.5 | 2.0 | Not available |
| #8 | 13.7 | 1.0 | 2.0 | 1.5 |
| #9 | 26.0 | 2.0 | 3.0 | 1.5 |
| #10 | 17.8 | 3.0 | 2.0 | Not available |
| #11 | 10.6 | 2.0 | 1.0 | 1.0 |
| Mean | 19.0 | 2.1 | 1.9 | 1.1 |
| SD | 8.8 | 1.2 | 0.8 | 0.4 |

intervals, could have anticipated this remodeling phase.

The present findings demonstrated that great variability exists in man with respect to hard tissue formation within extraction sockets. Thus, whereas a provisional connective tissue consistently forms within the first weeks of healing, the interval during which mineralized bone is laid down is much less predictable. Despite the high heterogeneity that was observed in the composition of the specimens within each observation interval, each tissue component shows a well-defined tendency to change over time. Apparently, the bone organization and architecture was not completed at 24 weeks after tooth extraction.

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**Clinical Relevance**

*Scientific rationale for the study*: Studies on post-extraction wound repair are affected by significant limitations and do not evaluate all compartments of the bone defect.

*Principal findings*: The process of remodeling, i.e. the replacement of woven bone with lamellar bone and marrow, is slow. Limited morphological alterations seem to occur between 6–8 and 12–24 weeks of healing. High inter-individual heterogeneity was noted within each observation interval.

*Practical implications*: The great variability observed in the rate of new bone formation from patient to patient may be of clinical relevance when to determine the most suitable time for implant placement in post-extraction sockets.