

Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. III: soft tissue findings

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Abstract

Aim: To describe histologically the early phases of soft tissue healing to implants placed into fresh extraction sockets.

Materials and Methods: In 16 beagle dogs, 64 3.25-mm-wide cylindrical screw implants were inserted into the distal sockets of the third and fourth lower premolars using a one-stage trans-mucosal healing protocol. Biopsies were then taken at 1, 2, 4 and 8 weeks and prepared for histological examination.

Results: One-week specimens showed a junctional epithelium and an underlying loose connective tissue rich in inflammatory cells. At 2 weeks, signs of epithelial proliferation and a more organized connective tissue were observed. At 4 and 8 weeks, inflammation was absent; the epithelium appeared mature and in close contact with the surface of the healing abutment or the implant. The connective tissue was dense in an area close to the implant surface and the fibres were aligned parallel to the implant surface. The soft tissue dimensions at 8 weeks were approximately 5 mm, including about 3–3.5 mm of epithelium and 1–1.5 mm of connective tissue.

Conclusion: Soft tissue healing to implants placed in fresh extraction sockets may result in a longer epithelial interface than implants placed in a healed ridge.

Key words: biological width; extraction socket; immediate implants; junctional epithelium; peri-implant mucosa

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The biological width is a well-defined anatomical concept that describes the dimensions of a soft tissue barrier around implants. It comprises a coronal epithelial portion measuring between 1.5 and 2 mm and a connective tissue portion between 1 and 1.5 mm (Berglundh et al. 1991). The development of this structure has proved to be inde-

pendent from the implant surgical technique, i.e., whether the implant is submerged or non-submerged during surgery (Ericsson et al. 1996, Abrahamsson et al. 1999). It is also unrelated to the type of implant, such as one- or two-piece implants (Abrahamsson et al. 1996). The early formation and development (from 2 h to 3 months) of this soft tissue barrier around implants placed in a healed ridge has been documented recently, corroborating previous results (Berglundh et al. 2007).

In recent years, immediate implant placement after tooth extraction has become a common surgical protocol. One of the controversies with this sur-

gical approach is whether the formation of a soft tissue barrier follows the same pattern as when placing dental implants in healed ridges. Araujo et al. (2005, 2006), in an experimental study in dogs, reported that the dimensions of the mucosal seal around implants immediately placed in fresh extraction sockets were comparable to those found around standard implants. In contrast, other experimental studies that compared healing at implants placed in a healed ridge with implants immediately placed in fresh extraction sockets have reported larger soft tissue dimensions in implants placed in extraction sockets (Schultes & Gaggl 2001). Similar results were

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reported in a recent experimental study in minipigs (Rimondini et al. 2005). In light of these contradictory results, the present experimental study in the dog was performed with the aim of describing the early healing phases of soft tissue healing to implants placed in fresh extraction sockets.

Materials and Methods

The experimental model used in this experimental study was recently described in an earlier publication (see Vignoletti et al. 2009a, b). Briefly, 3.25-mm-wide cylindrical screw implants (Osseotite Certain, Biomet 3i, Palm Beach Gardens, FL, USA) were inserted into the distal sockets of the lower third and fourth premolars and allowed to heal from 1 week to 8 weeks following a one-stage trans-mucosal surgical protocol.

This animal experimental study was carried out at the Experimental Surgical Centre of the Hospital "Gomez-Ulla" in Madrid, once the *Regional Ethics Committee for Animal Research* had approved the study protocol. Sixteen adult female Beagle dogs were included. Buccal and lingual intrasulcular incisions from the mesial aspect of the third premolars to the mesial aspect of the first molars were performed on both sides of the mandible. Mucoperiosteal full-thickness flaps were reflected on both sides to disclose the marginal aspect of the ridge in order to facilitate tooth extraction. The third and fourth mandibular premolars ($3P_3$ and $4P_4$) on both sides were hemisected and extracted. The distal socket of each two-rooted premolar was selected as the study site, while the mesial sites were allowed to heal without intervention (Fig. 1).

Osteotomy preparations were made to the appropriate diameter into the centre of the sockets ensuring that the implant shoulder was placed at the level of the marginal portion of the buccal plate



Fig. 1. The distal sockets of third and fourth mandibular premolars were elicited as implant study sites.

(Fig. 2). Healing abutments were connected and the flaps were repositioned and sutured with 4-0 vicryl resorbable sutures in order to allow a transmucosal healing (Fig. 3). The animals were sacrificed and biopsies were obtained; thus, specimens for four healing periods from 1 week to 8 weeks after implant installation were provided.

Histological processing

At each biopsy interval, the animals were sacrificed with an overdose of sodium-pentothal and perfused with a fixative solution (Karnovsky 1965) through the carotid arteries. Calcified ground sections were prepared according to the methods described by Donath & Breuner (1982) and in accordance to the protocol outlined by Vignoletti et al. (2009a, b).

Histological and histometric evaluation

The histometric evaluation was carried out in a Leitz DM-RBE microscope (Leica, Heidelberg, Germany) equipped with image analysis software (Q-500 MC; Leica).

Four bucco-lingual sections per animal were analysed and the following landmarks were identified on the buccal and lingual side of the implants:

- PM, the margin of the peri-implant mucosa.
- aJE, the apical border of the junctional epithelium.
- B, the most coronal position of bone-to-implant contact.

Linear distances between the landmarks were measured and expressed in millimetres.

Data analysis

Data from the 1, 2, 4 and 8 weeks of healing were evaluated and compared.



Fig. 2. The implant shoulder was levelled with coronal bone crest on the buccal aspect.



Fig. 3. Flaps were sutured, allowing a transmucosal healing.

The dog was used as the statistical unit of analysis. For each variable, a mean value for each animal and healing interval was calculated and used for the data analysis. Histometric results were expressed in mean linear distances of buccal and lingual measurements (\pm SD). The one-way analysis of variance test with the Bonferroni post hoc analysis was used to evaluate differences among groups. Differences were considered statistically significant when p was <0.05 . The statistical analysis was performed using the software PRISM 5.0 (Graph Pad, San Diego, CA, USA). Furthermore, at each healing interval, a mean value of each variable was calculated for implants placed in the third or the fourth premolar sites ($3P_3$ and $4P_4$). Because of the limited number of animals per group, no statistical analysis was performed on such stratified data and descriptive results were presented.

Results

Clinically, healing was uneventful for all the 64 implants and occurred without signs of inflammation in the peri-implant mucosa. Two healing abutments in one dog at 4 weeks and four healing abutments in two dogs at 8 weeks were lost, and consequently, these specimens were discarded from the histometric analysis. One implant in the third premolar site ($3P_3$) of one dog from group-2 (1 week) suffered a surgical complication during implant placement and a complete dehiscence at the buccal aspect of the socket occurred. The specimen from this implant was also excluded from the histometric analysis. The final number of specimens per group that were histologically evaluated is presented in Table 1.

Histological observations

A mismatch was often observed between the implant shoulder and the healing

Table 1. Number of animals per group, number of specimens analysed and mean (SD) dimension of the microgap

Healing interval	Number of animals	Number of specimens	Microgap [mean (SD) μ m]
1 week	3	11	127 (143)
2 weeks	3	12	321 (282)
4 weeks	3	10	346 (337)
8 weeks	4	12	116 (137)

abutment. The mean distance (SD) of this gap calculated at the buccal aspect of each implant is presented in Table 1.

Figure 4 depicts the peri-implant mucosa at 1 week. The tissue was lined by an oral epithelium (Fig. 4a) that was continuous with the junctional epithelium. In some cases, the epithelium was in contact with the most coronal portion of the implant surface (Fig. 4b). The connective tissue was infiltrated with inflammatory cells. The bone tissue was comprised of bundle bone and lamellar bone in different proportions depending on the width of the bone plate. Numerous osteoclasts were present at the inner and the outer portion of the crest (Fig. 4c).

At 2 weeks, inflammation was still present. The epithelium was comprised of multiple layers of cells in its coronal portion, while in the most apical part it was in close contact with the titanium surface with only a few cell layers. (Figs 5a and b). The connective tissue comprised many elongated fibroblast-like cells that were aligned parallel to the implant surface and separated from the implant surface by only a few cell layers (Fig. 6). Lateral to this area, many small vessels and connective tissue fibres were observed (Fig. 7).

At 4 and 8 weeks, inflammation was absent. The barrier epithelium appeared mature and was in close contact to the titanium surface of the implant or the healing abutment (Fig. 8). In some sites, the epithelium extended to the implant threads close to the newly formed bone (Figs 9 and 10). The part of the supra-crestal connective tissue that was close to the implant surface was dense and rich in fibroblasts. In the lateral portion, collagen fibres ran mostly in a direction parallel to the implant surface in a richly vascularized connective tissue matrix (Figs 7 and 8).

Histometric analysis

The results from the histometric measurements (mm) are shown in Tables 2–4.

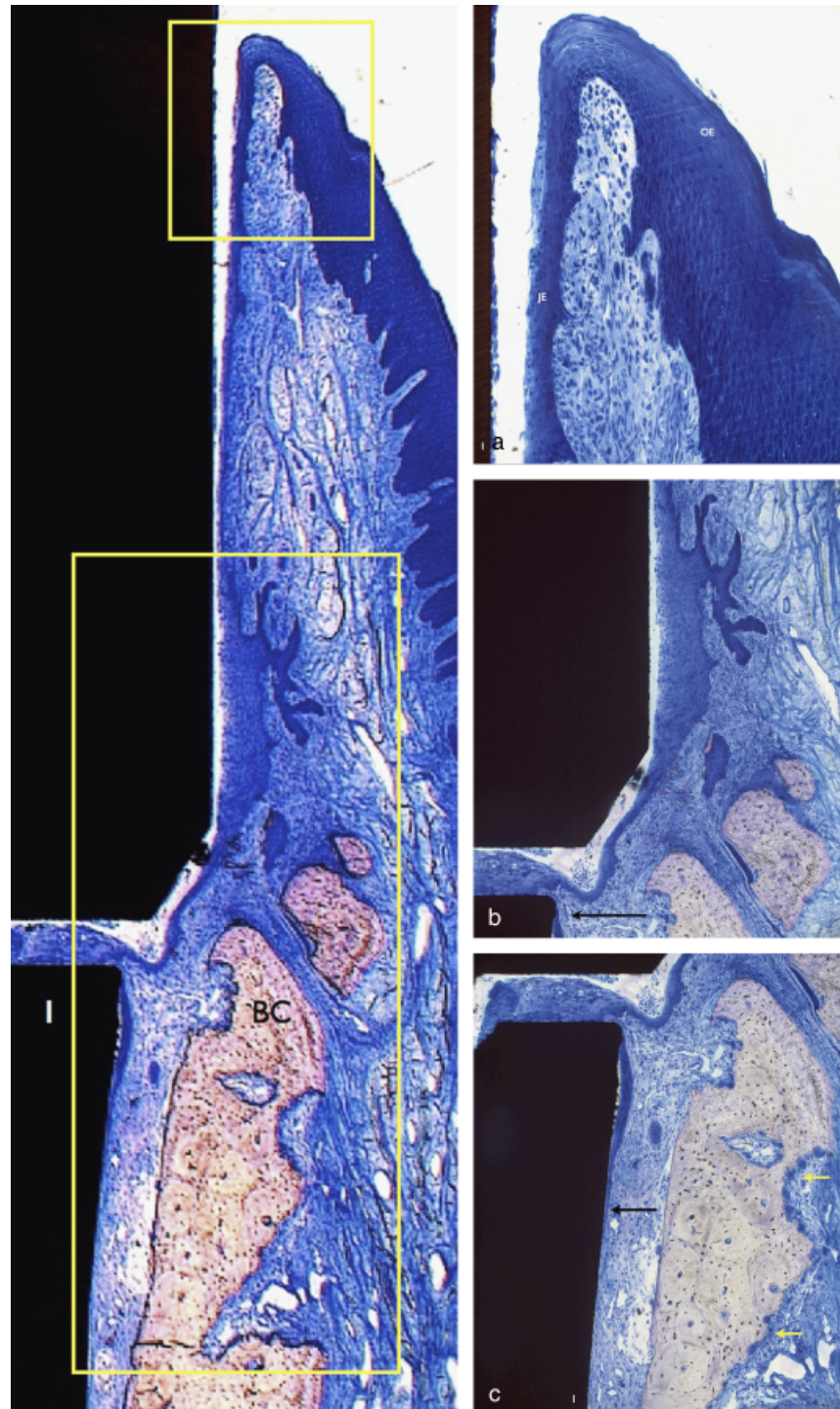


Fig. 4. Implant and surrounding tissue after 1 week of healing. A junctional epithelium is interposed between the implant surface (I) and the thin buccal bone crest (BC). Toluidine blue staining. Original magnification $\times 2.5$. Insets: (a) The oral epithelium (OE) is continuous with the junctional epithelium (JE) that is facing the implant abutment (I). Inflammatory cells are present. Toluidine blue staining. Original magnification $\times 10$. (b) Implant abutment interface. A microgap is present. The junctional epithelium and the connective tissue are infiltrated with inflammatory cells. Note the junctional epithelium in contact with the implant shoulder (arrow). Toluidine blue staining. Original magnification $\times 10$. (c) Most apical portion of the junctional epithelium (arrow) in intimate contact with the implant surface (I). Note the osteoclasts on the buccal bone crest (yellow arrows). Toluidine blue staining. Original magnification $\times 10$.

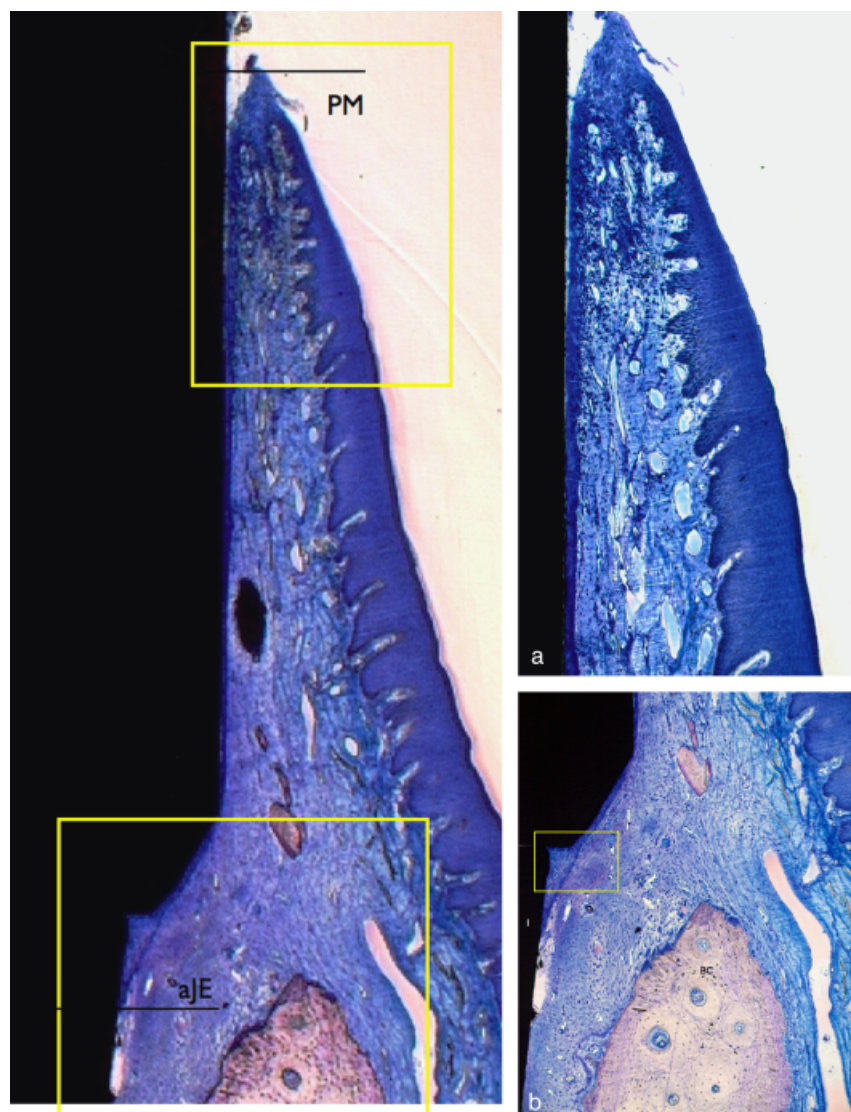


Fig. 5. Implant and surrounding tissue after 42 weeks of healing. Note the dimension of the junctional epithelium (PM-aJE). Insets: (a and b). Toluidine blue staining. Original magnification $\times 2.5$. (a) Inflammation is still present in the coronal portion of the periimplant mucosa. Toluidine blue staining. Original magnification $\times 10$. (b) A mature and dense connective tissue separates the bone crest (BC) from the implant surface (I) Inset: Fig. 6. Toluidine blue staining. Original magnification $\times 10$.

PM-aJE (Table 2)

On the buccal aspect, the epithelium measured 2.35 (0.84) mm at 1 week, while at 2 weeks it extended to 3.06 (0.97) mm. At 4 weeks a rebound to baseline values was observed, whereas at the end of the study the mean position of the junctional epithelium was 3.34 (0.75) mm apical to the mucosal margin. The differences between 1 and 8 weeks and between 4 and 8 weeks were statistically significant ($p < 0.05$). On the lingual aspect, the epithelium was 1 mm shorter than at the buccal at 1 week, measuring 1.39 (0.85) mm. This difference persisted

at 2 weeks, while the 4-week data were similar between buccal and lingual aspects. At 8 weeks the most apical junctional epithelium was observed 2.79 (0.93) mm apical to the mucosal margin. The difference between 1 and 8 weeks was statistically significant ($p < 0.05$).

The overall dimension of the epithelium extended 1.87 (0.68) mm apical to the mucosal margin after 1 week of healing, whereas at 2 weeks the epithelium extended to 2.64 (0.58) mm. At 4 weeks, the dimension of the epithelium reverted towards the 1-week values, although at 8 weeks the mean epithelial dimension was 3.07 mm (Fig. 11).

aJE-B (Table 3)

The connective tissue portion, outlined by the distance from the most apical cell of the junctional epithelium (aJE) to the most coronal bone to implant contact, presented longer dimensions on the lingual side compared with the buccal after 1 week. The mean aJE-B distance measured 3.34 (1.83) and 4.52 (1.69) mm on the buccal and lingual sides, respectively. Although a marked difference was present at 1 week, similar dimensions were observed after 2 weeks of healing that averaged 2.40 mm. At 4 weeks, the connective tissue portion measured 2.27 (0.30) and 1.25 (0.36) mm on the buccal and the lingual aspects, respectively, whereas at the end of the study the supracrestal connective tissue reached rather similar dimensions on both aspects of the alveolar ridge. The most coronal bone to implant contact was located 1.57 (0.18) and 1.90 (0.71) mm apical to the junctional epithelium on the buccal and the lingual aspect, respectively. Differences between 1 and 8 weeks were statistically significant ($p < 0.05$) on the buccal aspect and between 1 and 8 weeks and between 1 and 4 weeks on the lingual aspect.

The overall dimension of the connective tissue portion averaged 3.93 (0.83) mm at 1 week. Two weeks after implant installation, this dimension decreased to 2.40 (1.02) mm. At 4 weeks, this supracrestal connective tissue dimension further decreased to 1.76 (0.71) mm and then remained stable from 4 to 8 weeks, averaging 1.74 (0.23) mm. The overall dimensional changes of this compartment showed a clear contraction from 1 week till the end of the study (Fig. 11).

PM-B (Table 4)

The soft tissue dimensions, calculated as the distance between the marginal peri-implant mucosa and the most coronal bone-to-implant contact, were similar between the buccal and the lingual aspect throughout the study.

At 1 week, the overall dimension of the soft tissues averaged 5.76 (2.65) mm (Table 4). There was then a gradual reduction from 1 to 4 weeks, changing from 5.76 to 3.72 (0.88) mm. From 4 to 8 weeks, however, the soft tissue barrier demonstrated a rebound towards baseline values (Fig. 11). Differences

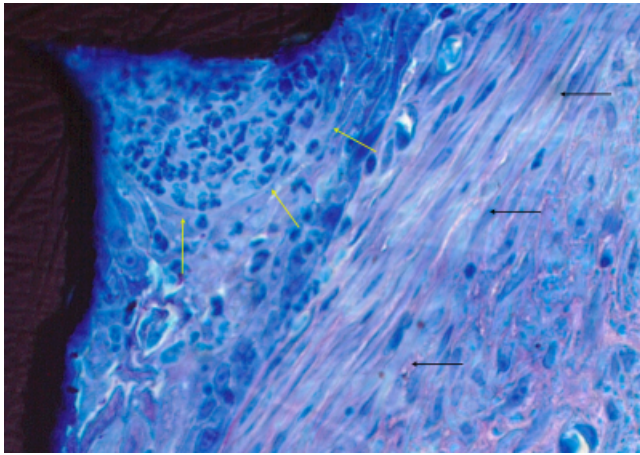


Fig. 6. Inflammatory cells (yellow arrows) are observed within the junctional epithelium at the implant–abutment interface. Note the densely packed connective tissue with absence of blood vessels, abundance of fibroblasts with thin collagen fibres. Fibroblasts are oriented with their long axis parallel to the implant surface (black arrows). Toluidine blue staining. Original magnification $\times 20$.

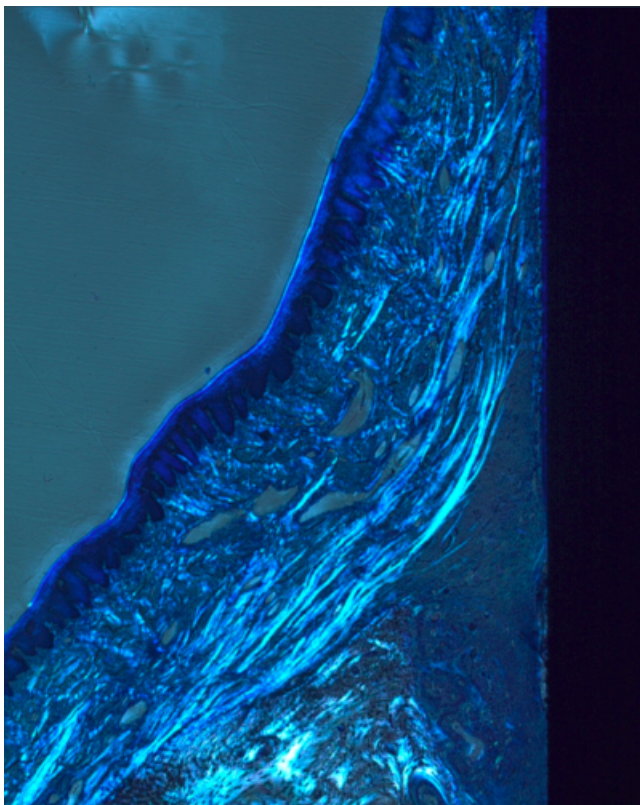


Fig. 7. Implant and surrounding tissues after 4 weeks of healing. Collagen fibres extend from the periosteum of the bone crest and run parallel to the implant–abutment surface. Polarized light. Toluidine blue staining. Original magnification $\times 20$.

between healing periods were not statistically significant.

Socket analysis

When dimensions were analysed according to socket type (the third premolar

versus the fourth premolar), a larger soft-tissue dimension was observed at the fourth premolar site at 4 and 8 weeks after implant installation. Although the dimensions of the epithelium were almost identical, the connective tissue component was higher at the fourth premolar socket.

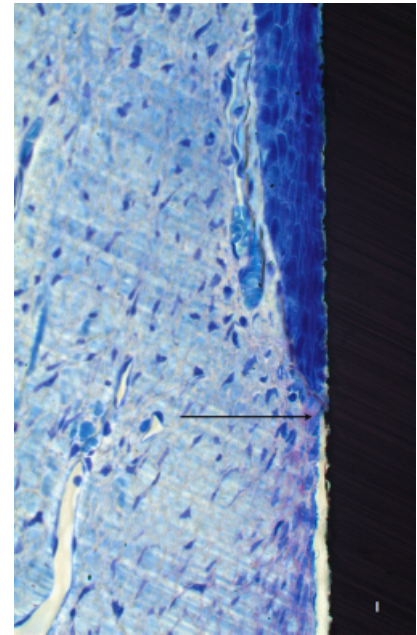


Fig. 8. Detail of Fig. 7. The junctional epithelium is in intimate contact with the abutment surface (I). Arrows indicate the most apical junctional epithelium. Toluidine blue staining. Original magnification $\times 10$.

Discussion

This study evaluated the formation and maturation of the soft tissues around implants immediately placed after tooth extraction, at 1, 2, 4 and 8 weeks of healing. The histometric analysis revealed a mean overall soft tissue dimension of 4.82 (0.16) mm at 8 weeks. This soft tissue barrier was comprised of a connective tissue portion that measured 1.74 (0.23) mm and an epithelial portion that measured 3.07 (0.39) mm. These findings are not consistent with the results reported by Berglundh et al. (1991) in an experimental study in the Beagle dog. These authors installed 3.75-mm-wide cylindrical implants in the edentulous mandibular premolar region, and 4 months after abutment connection, the dimensions of the biological width were evaluated. The overall mean dimension of the mucosal barrier was 3.80 (0.65) mm including a 1.66 (0.23) mm high connective tissue component and 2.14 (0.47) mm of epithelium.

In a recent study, the formation and maturation of the peri-implant mucosa from 2 h to 12 weeks around implants placed in a healed crest was reported from the same group (Berglundh et al. 2007). During the first 2 weeks of heal-

Table 2. Results of the histometric measurements of the junctional epithelium (mean and SD)

PM-aJE	Buccal Lingual	Buccal	Statistics	Lingual	Statistics
1 week	1,87 (0,68)	2,35 (0,84)		1,39 (0,85)	
2 weeks	2,64 (0,58)	3,06 (0,97)		2,23 (1,58)	
4 weeks	1,95 (0,16)	2,07 (0,20)		1,83 (0,26)	
8 weeks	3,07 (0,39)	3,34 (0,75)		2,79(0,93)	

PM, margin of the peri-implant mucosa; aJE, apical border of the junctional epithelium.

Table 3. Results of the histometric measurements of the connective tissue component (mean and SD)

aJE-B	Buccal Lingual	Buccal	Statistics	Lingual	Statistics
1 week	3,93 (0,83)	3,34 (1,83)		4,52 (1,69)	
2 weeks	2,40 (1,02)	2,33 (0,50)		2,47 (1,91)	
4 weeks	1,76 (0,71)	2,27 (0,30)		1,25 (0,36)	
8 weeks	1,74 (0,23)	1,57 (0,18)		1,90 (0,71)	

aJE, apical border of the junctional epithelium; B, most coronal position of bone to implant contact.

Table 4. Results of the histometric measurements of the peri-implant mucosa (mean and SD)

PM-B	Buccal lingual	Buccal	Statistics	Lingual	Statistics
1 week	5.76 (2.65)	5.58 (1.40)		5.95 (2.17)	
2 weeks	5.07 (0.44)	5.39 (1.90)		4.75 (2.40)	
4 weeks	3.72 (0.88)	4.34 (0.14)		3.09 (0.55)	
8 weeks	4.82 (0.16)	4.93 (0.63)		4.70 (0.51)	

PM, margin of the peri-implant mucosa; B, most coronal position of bone to implant contact.

ing, the barrier epithelium was located 0.5 mm apical to the marginal mucosa. Four weeks after implant placement, this distance increased to 1.4 mm and from 6 weeks it varied between 1.7 and 2.1 mm. These results are clearly different from the results presented in this study, where at 1 week the epithelium was about four times larger and this difference persisted throughout the study. One possible reason for these differences may be due to the different surgical implant protocols and therefore, the soft tissue healing may be different in implants placed immediately in extraction sockets. The finding of a longer epithelium when implants are placed in fresh extraction sockets has

also been reported by Rimondini et al. (2005). They evaluated the epithelial dimensions after placing implants in fresh extraction sockets in minipigs and reported that the epithelial length was 3.02 mm at 30–60 days after implant installation. These differences in the epithelial dimensions at implants placed in fresh extraction sockets may be due to the presence of a tooth-dependent epithelium that remained after extraction and became incorporated into the implant healing process.

Moreover, in the present experimental design, the mesial roots of the third and fourth premolars were extracted, thus creating a multiple extraction site. It has been documented that multiple

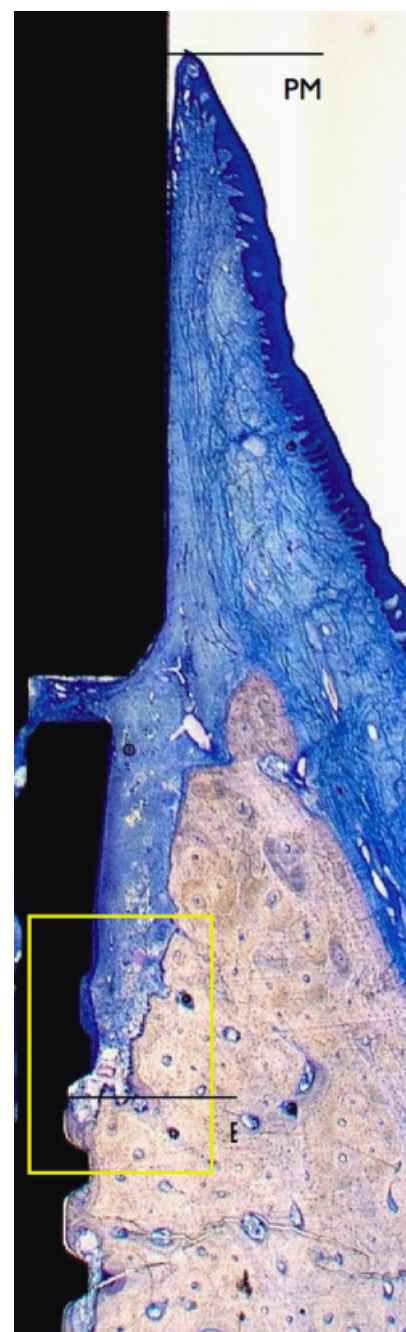


Fig. 9. Implant and surrounding tissues after 8 weeks of healing. Dimension of the soft tissue barrier (PM-B). Toluidine blue staining. Original magnification $\times 2.5$. Inset: Fig. 10.

extraction sites tend to produce more volumetric alterations than a single extraction site (Schropp et al. 2003), and therefore, a more pronounced horizontal and vertical bone resorption could also explain the longer soft tissue dimensions observed in the present experiment. Nevertheless, the amount of ridge alterations has been reported recently (Vignoletti et al. 2009a, b), and

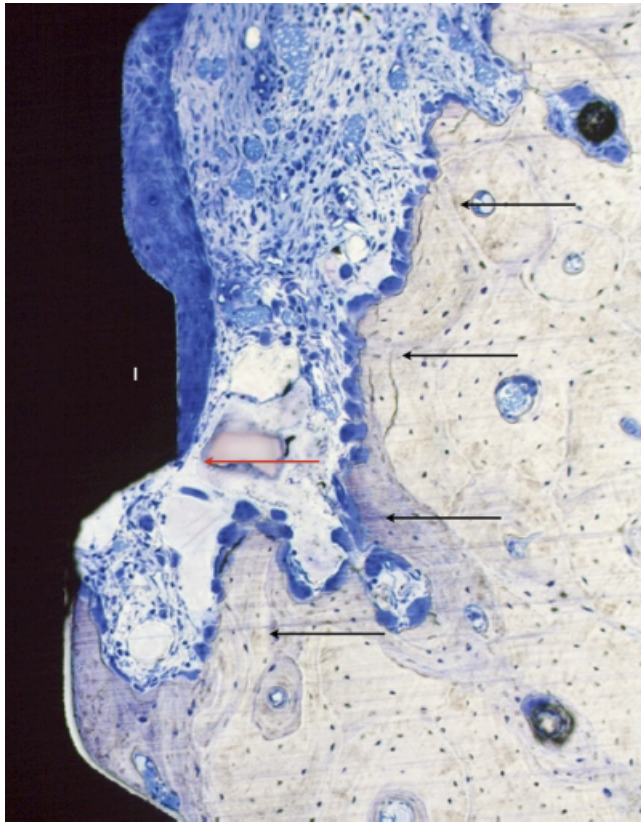


Fig. 10. Most apical extension of the junctional epithelium (arrow) occasionally observed on the implant threads (I). Note the line of osteoclasts (black arrows) on the bone surface. Toluidine blue staining. Original magnification $\times 20$.

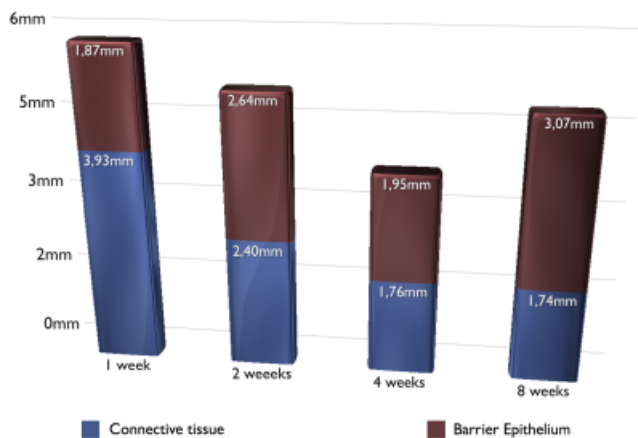


Fig. 11. Histogram illustrating the mucosal height from 1 to 8 weeks of healing. Epithelial (red) and connective tissue (blue) dimensions.

the results from the histometric measurements just showed a moderate mean resorption of the buccal bone plate [0.6 (0.7) mm], thus not justifying the longer dimensions of the junctional epithelium encountered.

Recently, it has been reported that a flapless approach at the time of tooth extraction may induce less volumetric

changes compared with a flapped surgical protocol (Fickl et al. 2008). In fact, Blanco et al. (2008), when studying ridge alterations following immediate implant placement with or without flap surgeries, demonstrated a longer soft tissue component in the flapped group. These findings, however, are not consistent with the histological data

reported recently by Araújo & Lindhe (2009), who demonstrated in a similar dog experiment that these differences between the two surgical protocols disappeared throughout a longer healing period. Although the question of whether flap elevation induces an additional trauma that influences the final dimensions of the alveolar process remains to be answered, we do not believe that the longer soft tissue dimensions observed in this study were due to the elevation of full-thickness flaps.

In this investigation, healing abutments could not fit precisely to the shoulder of the implant in some sites and, thereby, a mismatch occurred. This complication may be explained by either the subcrestal position of the implant shoulder on the lingual side or the slightly wider diameter of the healing abutments compared with the head of the implant (Fig. 5).

The mean dimension (SD) of this gap was calculated at the buccal aspect of each implant and included in the histometric analysis (Table 2). A micro-gap at the implant/abutment interface has been considered to be a potential factor influencing bone resorption in two-piece implants (Ericsson et al. 1996). In this investigation, there was no relation between the presence of the gap and the soft tissue dimensions (data not shown). Nevertheless, the misfit and the possible unstable condition of the healing abutments in some of the specimens should be taken into consideration when evaluating the histological outcomes.

In conclusion, this study demonstrated that the overall dimensions of the biological width around implants placed immediately after tooth extraction were approximately 5 mm, comprising a 3–3.5 mm high epithelial component and a 1–1.5 mm high zone of connective tissue. Future studies should validate these dimensions and further investigate the influence of this treatment protocol on the long-term maturation of the peri-implant mucosa and the possible clinical consequences.

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Clinical Relevance

Scientific rationale for the study: In recent years, immediate implant installation into fresh extraction sockets has become a common surgical protocol. Yet, limited information is available on (i) the dimensions and (ii) the development of the biological width around implants installed according to this therapeutic approach. Thus, the aim of this investigation was to describe the early phases of soft tissue healing to

implants placed immediately upon tooth extraction.

Principal findings: The soft tissue dimensions at implants placed immediately after tooth extraction are approximately 5 mm, including a 3–3.5 mm high epithelial component and a 1–1.5 mm zone of connective tissue. The junctional epithelium was approximately 1.5 mm at 1-week specimens and proliferated further up to 8 weeks of healing, while the connective tissue component exhib-

ited a gradual contraction throughout the study.

Practical implications: Findings from the present animal experiment are in contrast with data presented on implants placed in a healed ridge. It is suggested that the soft tissue healing may be influenced by the surgical protocol. The clinical consequences of a larger biological width are still unknown.

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