Histological Observations on the Healing of a Periodontal Defect Five Years after an Autogenous Bone Graft

by

P. MATTOUT*, E. KATCHBURIAN** and J. FOUREL***

(Received 22 February and accepted 11 March 1988)

Key words: juvenile periodontitis, periodontal healing, iliac autograft, new attachment

Abstract

A bone autograft obtained from the iliac crest was implanted into the periodontal region of the lower first molar (36) in a patient suffering from juvenile periodontitis. In the present paper we describe the results of a biopsy obtained 5 years after the graft implantation. The radiological, histological and ultrastructural images obtained suggested that neoformation of bone, cementum-like tissue, and of connective tissue interposed between the two, had occurred.

Introduction

A number of investigators have tried to obtain neoformation of the periodontium using bone autografts [1-11]. Postoperative observations suggested that bone neoformation was accompanied by neoformation of cementum and ligament [5,6,8,12]. Other studies have provided evidence indicating that migrating epithelial cells invade the periodontium and interpose themselves between the new bone and the root, thus preventing formation of a new ligament [13-16]. These observations were, however, limited to periods of less than one year after grafting. In the present paper we describe results obtained 5 years after implantation of an iliac crest autograft into the periodontium of the first lower molar (36) in a patient suffering from juvenile periodontitis.

Materials and Methods

Clinical

F.C., an 18-year-old female of French origin, was referred to the Department of Periodontology. On oral examination, a full permanent dentition was present and there was no evidence of active caries or defective restorations. However, both the lower and upper incisors and the first left molar (36) showed considerable mobility

* Faculté d'Odontologie, Service de Parodontologie, 21 Rue Sylbbelle, Marseille, France.
** The London Hospital Medical College, Dept. of Anatomy and Histology, Turner St, London, England.
*** Faculté d'Odontologie, Marseille Chef du Département de Parodontologie, Marseille, France.
Figs. 1 and 2
and deep periodontal pockets; bacterial plaque was virtually absent. There was severe bone loss around tooth 36, on the mesial side extending down to the furcation. Radiological examination confirmed the clinical findings and a diagnosis of juvenile periodontitis was made. An attempt was made to treat the periodontal osseous defects using an autogenous iliac marrow graft according to the technique previously described[17]. The patient was followed up for five years and satisfactory clinical and radiological results were obtained (Figs. 1 and 2). However, since a residual mesial bone defect remained, it was decided to perform a re-operation.

For this purpose, a full flap was raised to expose the area. Bone, similar in appearance to healthy alveolar bone, completely filled the furcation region but only partially filled the mesial aspect of the mesial root. At the buccal aspect, where a bone defect previously existed, supracrestal bone was present (Figs 1, 2 and insets). With the permission of the patient, a biopsy for histological examination was obtained during the operation to ascertain the extent of the healing in the region where clinical/radiological reconstruction was observed.

With the aid of a trepan measuring 2 mm in diameter a biopsy sample of the region of the mesial root of tooth 36 was obtained. The biopsy was confined to the site where the graft had been originally inserted and where bone neoformation was observed. The trepan was allowed to slightly penetrate the root surface, and then a fine probe inserted into the gap created by the trepan was used to remove in one single piece the biopsy sample containing a portion of the root surface, bone and interposed tissue.

**Histological**

The biopsy sample was fixed in a mixture of 4% glutaraldehyde and 4% formaldehyde, buffered at pH 7.4, at room temperature for 4 h. After osmification, the undecalcified specimen was dehydrated in a graded ethanol series, treated with propylene oxide, and embedded in Epon. Semithin sections (1-3 μm) were stained with toluidine blue. In view of the difficulty in sectioning and staining the mineralized tissues, most of our observations were carried out using Nomarski optics.

For electron microscopy, ultrathin sections were collected onto grids, and stained with lead citrate and uranyl acetate prior to examination in a JEOL-SEM 100 B electron microscope.

**Results**

Examination of the specimens obtained from the regions where the graft had been placed revealed the presence of periodontal elements. Mature lamellar bone showing osteocytes and layers of well calcified collagenous matrix was also clearly

---

**Fig. 1** Radiograph showing the original lesion-related tooth 36. There is considerable bone destruction in the proximal region as well as in the furcation zone. The inset shows the clinical aspect of the lesion.

**Fig. 2** Radiograph of the same region as in previous figure (Fig. 1). Five years after grafting, bone has filled the furcation zone almost completely; the proximal aspect also shows zone improvement. The inset shows the clinical aspect: bone similar in appearance and hardness (tested by probing) is present in the same region as observed in the radiograph.
observed (Figs. 3, 4 and 5). The periodontal surface of the bone showed an irregular profile, often exhibiting a fine layer of basophilic material, presumably osteoid. This surface was covered by a connective tissue layer with numerous cells (Figs. 3, and 4). Most of the cells near the bone surface were probably osteoblasts. With the aid of Nomarski optics, wavy strands (collagen bundles) of periodontal ligament-like tissue (Figs. 3, 4 and 8) and bone (Fig. 5) were observed. The sections permitted visualization of concentric layers of lamellar bone similar to haversian systems (Fig. 5). Ultrastructural examination of the specimens was limited in view of the difficulty in obtaining sections. However, they revealed the presence of numerous collagen fibrils in the periodontal ligament-like tissue (Fig. 9). In the newly formed bone, well mineralized collagen fibrils were present (Fig. 6).

In some regions, it was possible to observe a thin layer of cementum-like tissue apposed to the root surface (Fig. 7). Where it was possible to visualize the bone and the root in the same section, there appeared to be a continuous layer of connective tissue running from one surface to the other, similar to periodontal ligament (Fig. 8).

Discussion

Our clinical and radiological results showed that complete filling of the inter-radicular lesion and partial filling of the mesial defect had occurred. There was

---

**Fig. 3** A histological section of the periodontal surface of the newly formed bone. The surface of the bone shows a distinct basophilic layer, presumably osteoid (arrows). Connective tissue cells (c) are present in the region on the ligament (L); B, Bone; S, Osteocytes. Plastic section stained with toluidine blue. Nomarski optics.

$\times 410$

**Fig. 4** A section showing another region, similar to that in Fig. 3. The periodontal surface of the bone is extremely irregular (arrows). Connective tissue cells and presumably osteoblasts are present in close proximity to the bone surface. B, Bone; L, Ligament. Plastic section stained with toluidine blue. Nomarski optics.

$\times 550$

**Fig. 5** A section showing concentric layers of lamellar bone around a central canal (A), similar to a haversian system.

$\times 600$

**Fig. 6** Electron micrograph of the newly formed bone. Mineralized collagen fibrils (F) are present. Lead/uranyl staining.

$\times 23,000$

**Fig. 7** Histological section of the interface between the dentine and the ligament. A layer of newly formed tissue resembling cementum (arrows) is present between the dentine (D) and the ligament area (L). Plastic section stained with toluidine blue. Nomarski optics.

$\times 850$

**Fig. 8** Histological section showing newly formed connective tissue in the periodontal ligament area (L). A continuous layer of connective tissue runs from the root (D) to the bone surface (B). A layer of tissue resembling cementum (X), similar to that in Fig. 7, is present on the surface of the root. Plastic section stained with toluidine blue. Nomarski optics.

$\times 625$

**Fig. 9** Electron micrograph of the region of the periodontal ligament. Numerous wavy collagen fibrils (F) are present. Lead/uranyl staining.

$\times 15,000$
formation of supracrestal bone in the vestibular region of the proximal root, which had been originally devoid of bone. Intraosseous filling of pockets has already been reported\(^{[6,7,12,18]}\), but to the best of our knowledge, complete filling has rarely been described in interradicular lesions. The deposition of new bone in the supracrestal region, observed in the proximal root, is also a rare observation.

Since in humans it is not possible to make a histological reference notch on the root surface, as is usually done in experimental studies on animals, the interpretation of our results is limited.

Our histological results, as observed in plastic sections using Nomarski optics, confirm the presence of bone and periodontal elements. The bone was lamellar in nature and its periodontal face often exhibited an irregular profile, a basophilic appearance and a population of bone lining cells, which together are consistent with the interpretation that the bone surface was still undergoing remodelling. The space between the bone and the root showed numerous collagen fibers apparently running perpendicular to the bone surface. At the ultrastructural level, collagen fibrils were often seen to be twisted, possibly indicating imperfect collagen orientation in the newly formed periodontal region or, perhaps, an artefact due to torsional movement of the drill during the biopsy.

Our results showed a discernible cementum-like layer in the region of the newly formed periodontal region; no epithelial elements were found in this region. Thus, we believe that some form of attachment to the root surface had occurred. Previous studies in monkeys have shown that epithelial cells often migrate down to the site of the lesion and thus create a barrier for the formation of new ligament\(^{[19]}\). The present observations, obtained 5 years after grafting, suggest that the success obtained was due to the use of the iliac graft according to the procedure previously published\(^{[17]}\). It also appears necessary to allow the tissues and the graft to interact for a long period of time before any judgement can be made about the outcome. Most of the previous studies were conducted 6 or 7 months postoperatively\(^{[6,16]}\). The possibility that some of our observations may have been the result of spontaneous healing processes unrelated to the graft cannot be completely excluded. Listgarten\(^{[21]}\) described the progressive replacement of epithelium attachment by a connective tissue junction in rats. However, evidence has shown that supracrestal bone never forms spontaneously. Also, previous studies have demonstrated that elements of the periodontal ligament are capable of inducing the formation of new cementum\(^{[15,19]}\). Moreover, studies using periodontal ligament implants have shown that a new attachment is formed\(^{[8,20]}\), and more recently, it has been suggested that new cementum formation is dependent upon the connective tissue elements of the ligament\(^{[22]}\). Also, it has been demonstrated that new bone is produced through the influence of periodontal ligament elements\(^{[19,22]}\).

Although there seems to be no doubt that continuous remodelling of constituents of the periodontal ligament occurs in many periodontal diseases, we believe that our present results show that implantation of a bone graft into the affected sites in cases of juvenile periodontitis stimulates the formation of new bone and that this new bone provides a structural and perhaps inductive basis for the growth of constituents of the periodontal ligament. Induction of bone formation by bone
fragments has previously been demonstrated in a variety of conditions[7,11,23].

References